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Marine Fish Larvae Growth and Survival Effects of Density-Dependent Factors Spotted Seatrout ('Cynoscion nebulosus') and Lined Sole ('Achirus lineatus')

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MARINE FISH LARVAE GROWTH AND SURVIVAL

Effects of Density-Dependent Factors: Spotted Seatrout (*Cynoscion nebulosus*) and Lined Sole (*Achirus lineatus*)

by

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16. ABSTRACT

Standard culture methods were developed for larvae of two common marine fishes, the spotted seatrout Cynoscion nebulosus and the lined sole Achirus lineatus. Culture methods were described and the relationships of survival, growth and yield to temperatures, food concentrations and egg stocking densities were determined. Two different diets, a laboratory-cultured diet based on the rotifer Brachionus plicatilis, supplemented in some experiments with brine shrimp Artemia salina nauplii, and a net-collected zooplankton diet were compared. Survival rates at metamorphosis in excess of 50% could be routinely obtained for both seatrout and lined sole when food, stock and temperature levels were near optimum. Survival rates were equal on either a rotifer or zooplankton diet. Spotted seatrout larvae survived better and grew faster than lined sole larvae, and the differences were more pronounced when food concentrations were low. Feeding rates and rations also were estimated for each species as functions of age, size, food type and, in the case of seatrout, temperature, For both species, 28°C, 1000 per liter food levels and egg stocking densities that do not exceed 5.0 per liter are suitable for routine culture of larvae that could be used in aquatic toxicology research.

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FOREWORD

The Environmental Research Laboratory of the U.S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a great extent, this requires research on the tolerance of marine organisms and their life stages as well as of ecosystems to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

A current objective at ERLN is to develop and verify acute and chronic test methods to determine water quality criteria requirements for protection of indigenous marine life. This requires the capability for culturing the early life stages of marine animals from many phylogenetic groups, specific habitats and trophic levels. The number and diversity of test species must be adequate to support toxicological research providing the data base for developing national marine water quality criteria, site-specific criteria and standards, and marine permit programs such as estuarine and ocean waste disposal.

This study was accomplished as part of an overall effort to determine culture requirements for several species of fish suitable for use in toxicological studies. Standard methodology is provided for culturing the larval stages of two ecologically important marine fishes for application in bioassay experiments. The approach and methodology developed can be extended to additional species as appropriate. This helps to provide laboratory culture capabilities consistent with the needs for experimental organisms required for toxicological studies.

PREFACE

The larval stages of marine organisms are particularly sensitive to environmental stresses, but because of the difficulty in culturing and maintaining those stages it is hard to evaluate the potential effects of environmental contaminants on larval development. The purpose of this research was to develop and evaluate standard methodology to culture larvae of two common marine fishes, so that large numbers of healthy larvae or juveniles could be predictably produced for experimental research. In the report we document not only the methodology employed, but also survival and growth rates of larvae reared under different experimental conditions and other indices by which condition of the larvae can be assessed.

ABSTRACT

Research was undertaken to develop and standardize culture methods for larvae of two common marine fishes, the spotted seatrout Cynoscion nebulosus and the lined sole Achirus lineatus. Culture methods were described and the relationships of survival, growth and yield to temperatures, food concentrations and egg stocking densities were determined. Two different diets, a laboratory-cultured diet based on the rotifer Brachionus plicatilis, supplemented in some experiments with brine shrimp Artemia salina nauplii, and a net-collected zooplankton diet were compared. Survival rates at metamorphosis in excess of 50% could be routinely obtained for both seatrout and lined sole when food, stock and temperature levels were near optimum. Survival rates were equal on either a rotifer or zooplankton diet. Growth was faster on the zooplankton diet. Spotted seatrout larvae survived better and grew faster than lined sole larvae, and the differences were more pronounced when food concentrations were low. Growth rates of seatrout larvae were among the highest recorded for fish larvae. Feeding rates and rations also were estimated for each species as functions of age, size, food type and, in the case of seatrout, temperature. For both species, 28°C, 1000 per liter food levels and egg stocking densities that do not exceed 5.0 per liter are suitable for routine culture of larvae that could be used in aquatic toxiciology research. Despite considerable variability in survival and growth rates, it is possible to produce large numbers of larvae at any stage from hatching to metamorphosis for pollution bioassay research. Spotted seatrout larvae are easier to culture successfully than are lined sole, which might be a factor to consider in choice of a bioassay test organism.

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INTRODUCTION

Marine fishes can be routinely cultured through the larval stage, but there is a need to standardize culture procedures so that larvae in good physiological condition are available for pollution bioassays. Two species of common marine fishes that are found in coastal waters of the Gulf of Mexico and southeastern United States, the spotted seatrout (Cynoscion nebulosus) and lined sole (Achirus lineatus), were selected for this project to investigate how survival, growth and condition of the larvae were affected by culture methods. The roles of stocking density, prey concentration and prey type were investigated. Feeding rates of the larvae were determined as a function of age and in relation to the culture regime.

The ability to culture marine fish larvae for experimental purposes has increased greatly in the past ten years (Houde and Taniguchi 1979). Successful rearing techniques have not been standardized although there is a general recognition of important factors that affect both survival and condition of larvae. The role of food and nutrition in larval culture is most important (e.g. Styczynska-Jurewicz et al. 1979). Research results reported here focused on the roles of prey type and prey availability in predictably producing metamorphosed spotted seatrout and lined soles. In addition to aiding design of pollution and contaminant-oriented experiments, results of this study have helped to document environmental requirements of larvae of the two species.

CONCLUSIONS

Both spotted seatrout and lined sole can be routinely reared from hatching through metamorphosis on either a zooplankton or rotifer diet. Survival rates exceeding 50% from hatching to metamorphosis can be predictably obtained. Survival did not differ between the diets, but growth and yield were significantly higher on the zooplankton diet when equal food concentrations were compared. Survival, growth and yield can be predicted with reasonable confidence as a function of food concentration, but with less confidence as a function of stock density. At moderately high food concentrations, the degree of variability in survival and growth responses was similar on either the zooplankton or rotifer diet.

Spotted seatrout larvae survived better at 28° than at 24 or 32°C, but individual growth was best at 32°C. As stock densities increased, survival and growth of both spotted seatrout and lined sole decreased, but stock density effects were less defined than those of food concentration. Growth and survival increased as food levels were raised. Despite poorer survival and growth of individuals at high stock densitites, total yields of both seatrout and lined sole increased as stocking density was raised.

A laboratory-cultured diet of rotifers and Artemia salina nauplii was adequate to grow spotted seatrout and lined sole well into the juvenile stage with equal survival rates compared to fish fed zooplankton. Growth of spotted seatrout was relatively poor on rotifers-Artemia compared to zooplankton, but lined sole growth did not differ between diets. The Artemia component of the lab-cultured diet was responsible for the good growth of lined soles on that diet.

Eggs of spotted seatrout from plankton collections or from hormone-induced spawning gave equal survival and growth results in larval rearing experiments.

Growth of spotted seatrout larvae was faster than that of lined sole larvae at equal food concentrations. Specific growth rates of spotted seatrout fed zooplankton at 28°C ranged from 44.9 to 69.4% per day over the range of food concentrations that was fed, while lined sole specific growth rates ranged from 20.3 to 38.5% per day. Seatrout larvae growth rates were among the fastest reported for fish larvae, the maximum measured rate being 76.5% per day at 32°C and 1000 zooplankters per liter.

Feeding rates and rations were determined as functions of age and size of larvae as well as of food concentrations, and in the case of spotted seatrout the effect of temperature also was documented. Under the same conditions seatrout larvae consumed more food per unit time than did lined sole. Consumption of rotifers by 2-6 day-old seatrout larvae was higher than

zooplankton consumption, but subsequently zooplankton was consumed at higher rates, as larval predatory ability increased. Both seatrout and lined sole larvae could consume much more than 100% of their body weight per day when food concentrations were high.

Length-weight relationships were determined as functions of food concentration, food type (zooplankton or rotifers) and temperature for spotted seatrout larvae. Only temperature had a significant effect on the relationship; the fattest larvae were reared at 28°C. No significant difference was found in the length-weight relationship for lined sole reared at 28°C on zooplankton or rotifers at a 1000 per liter food concentration.

A routine culture procedure to provide spotted seatrout or lined sole larvae for aquatic toxicology research could be based upon 28°C rearing temperatures, 1000 per liter food concentrations (either zooplankton or rotifers) and a 5.0 per liter egg stocking density. Growth rates and survival rates of both species are higher at food levels exceeding 1000 per liter, but such levels are unlike any found in nature. If the goal of the toxicological study were to provide a large amount of assay material (i.e. yield), then egg stocking densities higher than 5.0 per liter could be used.

RECOMMENDATIONS

Information gained in this study can be used to standardize culture procedures from the embryonic to juvenile stage for spotted seatrout and lined sole larvae. Predictable growth and survival can be obtained using either a zooplankton or standard laboratory diet. The laboratory diet of rotifers (*Brachionus plicatilis*) and *Artemia* nauplii will be acceptable for use in toxicological studies. Both spotted seatrout and lined sole should be considered as candidates for test organisms in acute and chronic (partial life cycle) bioassays utilized in water quality and hazard substance evaluation research.

Further study is needed to understand nutritional requirements of spotted seatrout and lined sole larvae. Also, behavior of individual larvae in response to different feeding conditions needs to be determined. Indices of larval condition could be developed to help judge the physiological well-being of larvae reared under differing culture regimes. The recommended research would not only help advance rearing technology and standardization but would be useful in interpreting results of pollutant bioassay and environmental stress experiments.

METHODS AND PROCEDURES

GENERAL CULTURE METHODS

The general culture methods used to rear larval fishes at the University of Miami have been described in several published papers (e.g. Houde 1974, 1975, 1977). Houde and Taniguchi (unpublished report 1977) described in detail Miami culture methods. Their report is attached as Appendix A. Here, we give the general methods used for the EPA-supported research.

Obtaining Embryos

All embryos of lined sole and some seatrout embryos were obtained in 1-m diameter, 333 or 505-µm mesh plankton nets suspended from a dock in the tidal flow of Biscayne Bay. Embryos that were at the same developmental stage were pipetted from the plankton for experiments.

The usual method to obtain seatrout embryos was hormone-induced ovulation of captive females using human chorionic gonadotropin. The method was similar to published procedures (e.g. Stevens 1966; Shehadeh and Ellis 1970; Haydock 1971; Kuo *et al.* 1974). Details of the embryo obtaining procedure for spotted seatrout are given in Appendix B.

Culture Methods

Larvae were reared in semi-static 37.5 or 75-liter rectangular glass tanks with temperature controlled to $\pm$ 1.0°C by immersion heaters. A 13-hr light and 11-hr dark cycle was maintained. Salinities were held in the range 32 to 36°/oo, but usually were 33.5 to 35.0°/oo. In each experiment 10% of the tank volume was replaced with new seawater on day 3, 15% on day 5 and 20% on each subsequent odd day. Phytoplankton cultures, consisting of *Chlorella* sp. and *Anacystis* sp., were added and the blooms then maintained in rearing tanks. The role of the phytoplankton is not well understood but apparently it helps to keep nitrogen metabolites at low level (Alderson and Howell 1973; Cohen *et al.* 1976) and it may supplement the nutritional value of larval prey (Howell 1979; Scott and Middleton 1979). Fluorescent lights over the tanks provided 2500-4000 lux at the water surface.

Foods for larvae were zooplankton that was collected from Biscayne Bay in 53-µm mesh plankton nets, the cultured rotifer *Brachionus plicatilis* and nauplii of brine shrimp *Artemia salina*. The zooplankton was mostly nauplii and copepodites of the copepods *Oithona nana*, *Acartia tonsa* and *Paracalanus parvus*, the most common copepods in Biscayne Bay (Reeve 1970). Rotifers were cultured in 19-liter carboys, based on well-documented techniques (e.g. Ito 1960; Theilacker and McMaster 1971; Hirayama and Ogawa 1972; Hirayama *et al.* 1973; Howell 1973). Our rotifers were fed *Chlorella*; typical cultures at

25°C produced rotifers at densities of 200-300 per ml.

Both rotifers and zooplankton were graded through sieves to obtain food organisms of 50-110 µm width for initial feeding and 110-280 µm for older larvae. Food concentrations were determined by counting organisms in 50-200 ml aliquots pipetted from the rearing tanks at least three times daily. Food levels were adjusted when necessary by addition of food. When only the 50-110 µm fraction zooplankton or rotifers was being fed, the same numbers of the two kinds of food were maintained in their respective rearing tanks, because dry weights of individual prey were nearly equal. When the 110-280 µm fraction was added to the feeding schedule, the number of rotifers was maintained at twice the nominal food level to insure an approximately equivalent dry weight availability of rotifers to larvae for comparison with the tanks that were maintained on zooplankton at the nominal food level.

EXPERIMENTAL METHODS

Stock Density - Food Concentration Experiments

A range of embryo stocking densities and food level combinations was tested for seatrout and for lined sole. Each stock density - food level combination experiment was duplicated to provide a variance estimate. Two food types, zooplankton and rotifers, were tested. At the end of experiments survivors were counted, measured and dry weighed.

Spotted Seatrout

The experiments with zooplankton as food were run at 24, 28 and 32°C. Rotifer experiments were run at 28°C. Nominal food levels for each food type were 25, 100, 1000 and 5000 per liter. Embryo stock densities were 0.5, 5 and 25 per liter. The design provided 36 factor combinations for the zooplankton experiments and 12 combinations for the rotifer experiments. All experiments were of 12 days duration in 37.5-liter tanks.

Lined Sole

Experiments were run at 28°C until 16 days after hatching in 75-liter tanks. Nominal food levels for both zooplankton and rotifers were 50, 100 and 1000 per liter. Embryo stocking densities were 0.5, 2.0, 8.0 and 16.0 per liter. The design provided 12 factor combinations for each food type.

Rotifer-Artemia vs. Zooplankton Diets

Three replicated tests for lined soles and two for spotted seatrout, in which a diet consisting of rotifers and *Artemia* nauplii was compared to a zooplankton diet, were run for larvae reared at 28°C. Survival rates, lengths and weights were compared at 16 days after hatching for seatrout and 25 days after hatching for lined sole. The experiments were run in 37.5-liter tanks.

Spotted Seatrout

From day 2 to 6, larvae were fed on zooplankton (50-110 μm) or rotifers at 1000 per liter. From day 7 to 11 the zooplankton (110-280 μm) experiments were maintained at 500 per liter and the rotifer-*Artemia* experiments at 500 per liter rotifers and 500 per liter *Artemia*. From day 12 to 16 the zooplankton (> 280 μm) experiments continued at 500 per liter while the comparison diet was *Artemia* at 500 per liter. The experiment was run using two sources of eggs: hormone-induced and plankton net-collected to determine if egg source had an effect on survival or growth.

Lined Sole

From day 3 to 7, larvae were fed on zooplankton (50-110 μm) or rotifers at 1000 per liter. From day 8 to 12 the zooplankton (50-280 μm) experiments were maintained at 1000 per liter and the rotifer experiments at 2000 per liter. From day 13 to 20 zooplankton (110-280 μm) was maintained at 550 per liter while the comparison diet was rotifers at 1000 per liter plus *Artemia* at 550 per liter. From day 21 to 25 zooplankton (> 280 μm) levels were 500 per liter and the comparison diet was *Artemia* at 500 per liter.

Artemia Nauplii vs. Zooplankton Diet for Juvenile Lined Sole

Three replicates of an experiment were run at 28°C in which *Artemia* nauplii at 500 per liter were compared to zooplankton (> 280 μm) at 500 per liter for lined soles 13 to 25 days old. Prior to day 13 both treatment groups had been reared on zooplankton at 1000 per liter. Lengths and weight of samples of 10 individuals were obtained at days 13, 17, 21 and 25. Growth rates were estimated and differences in growth between diets were analyzed.

Growth Rates, Feeding Rates and Rations

Feeding rates and rations of both seatrout and lined sole larvae at several ages were estimated by transferring 3-30 larvae from their rearing tanks, which had been maintained at nominal food levels, to 10-liter tanks in which food levels were adjusted to the nominal levels. The reduction in number of food organisms in a 2-13 hr period (the time inversely related to larval age) allowed calculation of food consumption per larva per hr. Number of food items consumed per unit time was converted to weight consumed per unit time based on mean weights of organisms that were offered as food. Three replicated experiments were run for each larval age-food concentration combination.

Spotted Seatrout

Larvae from 2 to 12 days of age were tested using zooplankton as food at 24, 28 and 32°C and using rotifers as food at 28°C. Food levels were 25, 100 and 1000 per liter.

Lined Sole

Larvae from 3 to 17 days of age were tested at 28°C using zooplankton as

food at 50, 100 and 1000 per liter concentrations. The rotifer diet was to have been included in experiments, but EPA support for the project was withdrawn before such experiments could be run.

Length-Weight Relationships

Length-dry weight relationships were determined for spotted seatrout reared on zooplankton and on rotifers at each of the 25, 100, 1000 and 5000 per liter food levels. Effect of temperature on the length-weight relationship also was evaluated for seatrout reared on zooplankton. Length-weight relationships for lined sole reared at 28°C and at 1000 per liter food level were compared for the zooplankton and rotifer diets. The length-weight relationship was the power function $W = aL^b$, where W is larval dry weight, L is standard length and a and b are estimated coefficients.

Growth Rates and Survival Rates

Growth and survival of larvae from the stock density-food concentration experiments were determined from the numbers, lengths and weights of survivors at the end of experiments. Growth rates were estimated from lengths and weights of larvae that were tested in the feeding rate experiments. The rates were based on linear regressions of length at age data and on exponential regression model fits of weight on age data, $W_t = W_o e^{gt}$, where g is the instantaneous growth rate, W_t is estimated weight at age t (in days) and W_o is the estimated weight at hatching. Specific growth rates, 100 (antilog g−1), give the weight specific growth rate as a percent per day.

ANALYTICAL METHODS

Data analyses usually were based on regression and analysis of variance procedures. Multiple range testing methods were used when appropriate to determine significantly different mean values. All statistical tests were carried out with the probability level for rejection set at $\alpha = .05$. When necessary, weight and yield data were transformed to logarithmic values to homogenize variances among treatments. All percentage data were analyzed after arcsin transformation.

RESULTS AND DISCUSSION

STOCKING DENSITY AND FOOD CONCENTRATION EFFECTS

Both spotted seatrout and lined sole can be reared using either wild zooplankton or rotifers as food. Survival rates at metamorphosis increased as food concentrations were raised. Embryo stocking levels had a relatively small effect on survival, but lowest survival rates usually were observed at highest stock densities.

Spotted Seatrout

Taniguchi (1979) presented results of research on spotted seatrout larvae at the Early Life History Symposium, Woods Hole, including much of the EPA-supported stock density - food concentration study. An extended summary of that report (Taniguchi in press) will be published.

Percent survival over all factor combinations ranged from 0.0 to 84.7% for seatrout larvae reared on zooplankton and from 0.0 to 83.3% for larvae reared on rotifers (Table 1). On a zooplankton diet mean survival rate increased from 5.3 to 52.3% as food level was raised from 25 to 5000 per liter, a ten-fold increase in survival rate (Table 2). The differences in mean survival rates among food concentrations were highly significant (Table 3). A significant decrease in mean survival rate occurred at the 25.0 per liter stock density for larvae fed on zooplankton (Tables 2 and 3), although no difference in mean survival rate was found between the 0.5 and 5.0 per liter means. The zooplankton diet was fed at three temperatures (Table 1) and significant differences in survival occurred among temperature levels (Tables 2 and 3). Survival was significantly higher at 28^{o} than at 24 or 32^{o}C.

Survival of seatrout larvae that were fed rotifers did not differ significantly from survival of zooplankton-fed larvae (Tables 4 and 5). Mean survival rates over all food concentrations and stock densities were 37.9% for zooplankton-fed larvae and 43.6% for rotifer-fed larvae. If survival rate was the major criterion to judge culture success, either diet would be suitable. Survival of larvae increased significantly as food level was raised for both diets. Stock density effects were not significant at 28^{o}C (Table 5), mostly because there was no trend in mean survival rate for the rotifer-fed larvae (Table 4). Mean survival rates on rotifers were relatively high at the two lowest food levels, probably a result of relatively high capture success by first-feeding larvae preying on the slow-swimming rotifers.

Growth of seatrout larvae, expressed as standard length and dry weight, increased significantly as zooplankton concentrations and temperatures were

TABLE 1. SPOTTED SEATROUT. RESULTS OF REARING EXPERIMENTS AT 24, 28 and 32°C. EFFECTS OF DIET, TEMPERATURE, FOOD CONCENTRATION AND STOCK DENSITY ON SURVIVAL, GROWTH AND YIELD OF LARVAE AT 12 DAYS AFTER HATCHING. A ROTIFER DIET, BRACHIONUS PLICATILIS, WAS COMPARED TO A ZOOPLANKTON DIET AT 28°C, THE OPTIMUM TEMPERATURE.

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
I. Zooplankton Diet							
A. 24°C							
0.5	25	31	1	5.6	3.72	86.0	0.1
0.5	25	34	0	0.0	-	-	0.0
0.5	100	108	2	11.1	5.27	432.5	0.9
0.5	100	111	6	33.3	4.78	186.7	1.1
0.5	1000	1000	2	11.1	6.53	475.0	1.0
0.5	1000	1052	7	38.9	5.66	557.1	3.9
0.5	5000	5017	11	61.1	6.60	886.4	9.8
0.5	5000	4894	8	44.4	6.36	801.3	6.4
5.0	25	34	11	6.5	3.98	72.7	0.8
5.0	25	28	0	0.0	-	-	0.0
5.0	100	105	73	42.9	3.79	130.8	9.6
5.0	100	116	15	8.8	5.23	358.7	5.4
5.0	1000	1025	98	57.7	6.01	649.4	63.6
5.0	1000	1020	47	27.7	5.41	461.9	21.7
5.0	5000	4773	54	31.8	6.65	885.0	47.8
5.0	5000	5168	65	38.2	6.88	473.1	30.8
25.0	25	30	14	1.7	3.49	45.0	0.6
25.0	25	32	28	3.3	2.67	32.1	0.9
25.0	100	117	130	15.3	2.85	45.0	5.9
25.0	100	119	125	14.7	2.63	31.0	3.9
25.0	1000	932	472	55.5	5.20	421.6	199.0
25.0	1000	1124	300	33.3	4.51	249.5	74.8
25.0	5000	4325	393	46.2	6.87	295.9	116.3
25.0	5000	5045	112	13.2	5.43	528.2	59.2

(continued)

TABLE 1 (CONTINUED)

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
I. Zooplankton Diet							
B. 28°C							
0.5	25	28	1	5.6	4.64	210.0	0.2
0.5	25	22	7	38.9	6.04	621.4	4.4
0.5	100	102	10	55.6	7.26	798.0	8.0
0.5	100	101	10	55.6	6.44	589.0	5.9
0.5	1000	977	9	50.0	9.64	2350.0	21.2
0.5	1000	1060	10	55.6	9.50	2195.0	22.0
0.5	5000	4473	13	72.2	10.65	3497.7	45.5
0.5	5000	4949	11	61.1	10.99	3914.6	43.1
5.0		30	28	16.5	3.48	97.1	2.7
5.0	25	21	36	21.2	3.43	92.8	3.3
5.0	100	124	7	4.1	7.89	932.9	6.5
5.0	100	94	75	44.1	4.09	186.0	14.0
5.0	1000	831	97	57.1	7.56	1032.6	100.2
5.0	1000	886	104	61.2	7.37	1039.3	108.1
5.0	5000	4543	144	84.7	7.88	1927.2	277.5
5.0	5000	4471	135	79.4	8.61	1599.2	215.9
25.0	25	46	16	1.9	4.54	297.5	4.8
25.0	25	32	5	0.6	5.11	330.0	1.7
25.0	100	103	69	8.1	3.29	99.2	7.1
25.0	100	107	74	8.7	4.27	183.8	13.6
25.0	1000	984	360	42.4	5.72	554.4	199.6
25.0	1000	1061	346	40.7	4.92	329.1	113.9
25.0	5000	3549	572	67.3	6.60	807.6	462.0
25.0	5000	5052	412	48.5	7.27	1182.8	487.3

(continued)

TABLE 1 (CONTINUED)

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
I. Zooplankton Diet							
C. 32°C							
0.5	25	33	2	11.1	7.54	1170.0	2.3
0.5	25	41	2	11.1	6.50	700.0	1.4
0.5	100	115	8	44.4	8.64	1701.3	13.6
0.5	100	111	5	27.8	8.36	1572.0	7.9
0.5	1000	1286	14	77.8	11.65	4423.6	61.9
0.5	1000	1088	4	22.2	12.63	5372.5	21.5
0.5	5000	4726	15	83.3	13.57	7082.0	106.2
0.5	5000	4568	8	44.4	13.21	6310.0	50.5
5.0	25	44	0	0.0	-	-	0.0
5.0	25	34	26	15.3	3.86	103.1	2.7
5.0	100	139	13	7.7	8.29	1356.9	17.6
5.0	100	106	56	32.9	4.64	210.5	11.8
5.0	1000	1152	3	1.8	11.25	3960.0	11.9
5.0	1000	1000	85	50.0	9.78	2484.6	211.2
5.0	5000	4763	107	62.9	11.50	4091.7	437.8
5.0	5000	4529	55	32.4	12.09	4777.6	262.8
25.0	25	40	6	0.7	6.05	541.7	3.2
25.0	25	28	28	3.3	4.11	136.4	3.8
25.0	100	108	45	5.3	6.09	621.3	28.0
25.0	100	127	35	4.1	5.55	520.0	18.2
25.0	1000	1121	16	1.9	9.57	2558.1	40.9
25.0	1000	1072	109	12.8	9.01	1928.3	210.2
25.0	5000	5578	274	32.2	8.92	1925.6	527.6
25.0	5000	4724	284	33.4	9.38	2113.9	600.3

(continued)

TABLE 1 (CONTINUED)

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
II. Rotifer Diet							
A. 28°C							
0.5	25	167	14	77.8	4.20	210.7	3.0
0.5	25	41	0	0.0	-	-	0.0
0.5	100	361	10	55.6	3.94	92.0	0.9
0.5	100	309	1	5.6	6.17	540.0	0.5
0.5	1000	2513	12	66.7	4.46	132.5	1.6
0.5	1000	957	0	0.0	-	-	0.0
0.5	5000	9549	15	83.3	4.84	332.7	5.0
0.5	5000	9852	10	55.6	5.13	325.0	3.3
5.0	25	122	62	36.5	3.91	125.0	7.8
5.0	25	111	29	17.1	3.04	41.0	1.2
5.0	100	160	86	50.6	3.98	142.0	12.2
5.0	100	343	84	49.4	3.80	119.1	10.0
5.0	1000	1450	85	50.0	3.97	112.5	9.6
5.0	1000	2426	126	74.1	4.20	118.6	14.9
5.0	5000	7835	94	55.3	4.95	308.9	29.0
5.0	5000	9675	124	72.9	4.30	134.5	16.7
25.0	25	113	17	2.0	2.90	47.7	0.8
25.0	25	50	368	43.3	2.74	26.4	9.7
25.0	100	227	366	43.1	3.67	86.2	31.6
25.0	100	290	406	47.8	3.23	59.8	24.3
25.0	1000	1924	313	36.8	3.92	129.2	40.4
25.0	1000	1478	474	55.8	3.48	72.8	34.5
25.0	5000	7744	645	75.9	3.80	126.4	81.5
25.0	5000	6563	596	70.1	3.86	107.4	64.0

TABLE 2. SPOTTED SEATROUT. MEAN PERCENT SURVIVALS, LENGTHS, DRY WEIGHTS AND TOTAL YIELDS OF LARVAE REARED ON ZOOPLANKTON IN MULTIFACTOR EXPERIMENTS TESTING EFFECTS OF TEMPERATURE, FOOD CONCENTRATION AND STOCK DENSITY. THE VALUES AND STANDARD ERRORS ARE MEANS OVER ALL FACTOR COMBINATIONS FOR EACH FACTOR LEVEL.

	Percent survival		Standard length (mm)		Dry weight per larva (µg)		Total yield (mg)	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Temperature								
24°	21.2	1.4	5.17	0.40	336.1	74.8	3.3	1.9
28°	37.9	2.0	6.34	0.68	848.9	280.4	21.4	1.5
32°	21.5	1.9	8.96	0.85	2211.2	545.0	19.3	1.8
Food Concentration								
25	5.3	0.8	3.87	0.47	156.0	96.5	0.4	2.0
100	21.1	1.2	4.07	0.65	235.5	155.9	7.5	1.2
1000	36.5	1.6	5.93	0.55	662.2	314.7	43.0	1.4
5000	52.3	1.1	7.81	0.58	1416.6	494.2	106.8	1.4
Stock Density								
0.5	35.8	1.7	9.07	0.74	2641.5	504.6	4.0	1.7
5.0	28.6	2.1	7.24	0.83	1397.0	359.3	10.9	2.0
25.0	16.6	1.5	6.15	0.58	726.4	175.3	28.6	1.6

TABLE 3. SPOTTED SEATROUT. RESULTS OF THREE-FACTOR ANALYSIS OF VARIANCE EXAMINING EFFECTS OF TEMPERATURE, FOOD CONCENTRATION AND STOCK DENSITY ON FOUR VARIABLES FOR LARVAE FED ZOOPLANKTON. LARVAE WERE REARED IN DUPLICATED EXPERIMENTS TO 12 DAYS AFTER HATCHING AT FACTOR COMBINATIONS OF 24, 28 AND 32°C, 25, 100, 100 and 5000 PREY $LITER^{-1}$, AND 0.5, 5 AND 25 $LITER^{-1}$ STOCK DENSITIES.

Source	df	Mean percent survival calculated F	Mean standard length calculated F	Mean larval dry weight calculated F	Mean total yield calculated F
Temperature, T	2	7.05**	107.77**	88.05**	34.59**
Food Concentration, P	3	28.83**	91.29**	88.70**	162.44**
Stock Density, S	2	7.97**	42.60**	34.01**	37.53**
T, P	6	0.88	4.79*	0.61	2.05
T, S	4	2.07	3.36*	1.82	1.90
P, S	6	0.38	2.31*	2.86*	4.43*
T, P, S	12	0.74	1.20	1.36	0.91
Error	36	33	33	36	

* Significant at the .05 level

** Significant at the .01 level

TABLE 4. SPOTTED SEATROUT. EFFECT OF A ZOOPLANKTON OR ROTIFER DIET ON MEAN PERCENT SURVIVAL, MEAN STANDARD LENGTH, MEAN DRY WEIGHT AND MEAN TOTAL YIELD OF LARVAE REARED AT 28°C. THE MEAN AND STANDARD ERROR ARE GIVEN FOR EACH VARIABLE.

	Percent survival		Standard length (mm)		Dry weight (µg)		Total yield (mg)	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
I. Zooplankton Food								
28°								
Food Concentration								
25	10.9	2.1	3.94	0.40	183.1	80.1	2.1	1.6
100	26.0	3.6	4.25	0.78	223.6	145.6	8.6	1.2
1000	51.2	0.3	5.88	0.78	609.9	342.9	69.7	1.5
5000	69.5	1.1	7.25	0.73	1189.8	517.1	172.6	1.6
Stock Density								
0.5	48.1	1.9	8.80	0.83	2113.5	503.4	8.9	1.9
5.0	44.6	3.9	7.00	0.78	1163.3	246.6	29.3	2.0
25.0	22.4	3.7	6.03	0.45	695.6	128.0	37.5	2.2
II. Rotifer Food								
28°								
Food Concentration								
25	23.2	6.1	2.95	0.29	45.7	34.7	0.8	4.0
100	40.3	2.0	3.53	0.42	83.4	74.3	6.0	2.0
1000	42.7	5.4	3.76	0.16	100.0	10.7	2.6	5.2
5000	69.3	4.6	3.96	0.23	134.4	44.8	18.6	1.7
Stock Density								
0.5	35.5	7.5	4.54	0.33	229.7	66.8	0.3	3.5
5.0	50.6	1.4	4.14	0.19	146.9	26.8	9.7	1.4
25.0	45.0	2.7	3.56	0.16	90.1	13.2	21.4	1.7

TABLE 5. SPOTTED SEATROUT. RESULTS OF THREE-FACTOR ANALYSIS OF VARIANCE EXAMINING EFFECTS OF FOOD TYPE, FOOD CONCENTRATION AND STOCK DENSITY ON FOUR VARIABLES FOR LARVAE FED ZOOPLANKTON AND ROTIFERS. LARVAE THAT WERE FED ZOOPLANKTON WERE REARED IN DUPLICATED EXPERIMENTS TO 12 DAYS AFTER HATCHING AT FACTOR COMBINATIONS OF 24, 28 AND 32°C, 25, 100, 1000 AND 5000 PREY LITER^{-1} AND 0.5, 5 AND 25 LITER^{-1} STOCK DENSITIES. LARVAE THAT WERE FED ROTIFERS, BRACHIONUS PLICATILIS, WERE REARED IN DUPLICATED EXPERIMENTS AT 28°C FOR THE SAME PREY CONCENTRATION AND STOCK DENSITY FACTOR COMBINATIONS.

Source	df	Mean percent survival calculated F	Mean standard length calculated F	Mean larval dry weight calculated F	Mean total yield calculated F
Food Type, R	1	0.54	119.99**	124.95**	52.99**
Food Concentration, P	3	9.10**	25.31**	22.91**	70.08**
Stock Density, S	2	1.17	28.09**	20.04**	52.20**
R, P	3	0.60	10.94**	5.29**	15.27**
R, S	2	1.84	4.33*	.32	4.64*
P, S	6	0.37	1.47	.95	3.21*
R, P, S	6	0.54	2.05	1.97	1.10
Error	24	22	22	24	

* Significant at the .05 level

** Significant at the .01 level

raised (Tables 2 and 3). Lengths and weights of larvae decreased as stock density increased. Zooplankton-fed larvae at 12 days after hatching weighed 6.6 times more at 32° than at 24°C. Mean weights increased by a factor of 9.1 as zooplankton level was raised from 25 to 5000 per liter and decreased by a factor of 3.6 as stock level was raised from 0.5 to 25.0 per liter.

Growth increased as food concentration was raised for larvae reared on the rotifer diet and decreased as stock density increased, but the effects were not as clear as for zooplankton-fed larvae (Table 4). Larvae reared at 28°C grew better when fed zooplankton than when fed rotifers (Tables 4 and 5). At the 1000 per liter food concentration mean dry weights of seatrout larvae were 6.1 times higher on the zooplankton diet than on the rotifer diet.

Spotted seatrout larvae apparently survive well on a rotifer diet but they grow relatively slowly. Slow growth is not necessarily equated with poor physiological condition in seatrout larvae, but if fast growth is desirable in experiments, food other than rotifers should be provided. Our rotifers were fed on Chlorella and the rearing tanks contained blooms of phytoplankton so that either rotifers or copepod nauplii presumably were well nourished when consumed by the larval fish. It is possible that rotifers cultured in other ways would be more nutritious and could provide better growth to larvae. However, the great differences in growth of seatrout larvae that we observed lead us to believe that if fast growth is an important criterion in experiments, zooplankton will be a superior food.

Total dry weight yields of surviving seatrout at 12 days after hatching increased significantly as temperature, zooplankton concentration and stock density were raised (Tables 2 and 3). Effects were similar on both the zooplankton and rotifer diets (Tables 4 and 5), although total yields of zooplankton-fed larvae were significantly higher than yields of rotifer-fed larvae. For example, at the 5000 per liter food concentration, mean yield on zooplankton was more than nine times higher than that on rotifers. The difference was less extreme at lower food levels (Table 4), causing a significant food type by food concentration interaction (Table 5). In the case of zooplankton-fed larvae, it is important to note that mean total yields increased as stock level was raised despite the large decreases in mean weights and lengths of individual survivors (Table 2).

Predicability: For zooplankton-fed seatrout larvae, differences among temperature levels and food concentration levels, over all factor combinations, gave mean responses for survival, length and total yield that overlapped only a little when .90 confidence limits were calculated (Table 6). Stock density effects were less distinct and there was considerable overlap of confidence limits between stock density levels. Effects of temperature were most predictable for survival rate and least predictable for total yields. Each zooplankton concentration gave a mean survival rate with .90 confidence limits that did not overlap other zooplankton level mean survival rates. Mean total yields also were distinct among food concentrations for the zooplankton-fed larvae. There was no indication that variability in responses to any of the factors changed greatly as levels of the factors changed (Table 6).

TABLE 6. SPOTTED SEATROUT. NINETY PERCENT CONFIDENCE INTERVALS ABOUT THE MEANS FOR PERCENT SURVIVAL, STANDARD LENGTH, DRY WEIGHT PER LARVA AND TOTAL YIELDS OF LARVAE REARED IN THE LABORATORY AT THREE TEMPERATURES, FOUR FOOD CONCENTRATIONS AND THREE STOCKING DENSITIES USING ZOOPLANKTON AS FOOD. CONFIDENCE LIMITS ARE PLACED ON MEANS OVER ALL FACTOR COMBINATIONS AT THE DESIGNATED FACTOR LEVEL.

Factor and level	.90 CONFIDENCE LIMITS			
	Percent survival	Standard length (mm)	Larval dry weight (µg)	Total yield (mg)
Temperature				
24°	19.5 to 22.9	4.48 to 6.89	207.4 to 464.8	<0.1 to 6.6
28°	36.2 to 39.6	5.17 to 7.51	368.3 to 1329.5	18.8 to 24.0
32°	18.2 to 24.8	7.51 to 10.42	1275.4 to 3147.0	16.2 to 22.4
Food Concentration				
25	3.9 to 6.7	3.04 to 4.70	0.0 to 325.9	0.0 to 3.9
100	19.0 to 23.2	2.94 to 5.20	0.0 to 506.8	5.4 to 9.6
1000	33.7 to 39.3	4.97 to 6.89	114.6 to 1209.8	40.6 to 45.4
5000	50.4 to 54.2	6.80 to 8.82	556.7 to 2276.5	104.4 to 109.2
Stock Density				
0.5	32.9 to 38.7	7.80 to 10.34	1773.1 to 3509.9	1.1 to 6.9
5.0	25.0 to 32.2	5.81 to 8.67	780.1 to 2013.9	7.5 to 14.3
25.0	14.0 to 19.2	5.16 to 7.14	425.9 to 1026.9	25.9 to 31.3

Effects of the food concentration and stock density levels on mean responses were not as well defined for the rotifer diet as for the zooplankton diet. Based on the .90 confidence limits for factor levels used, only total yields relative to stock density differed enough to easily detect factor level effects (Table 7). Variability in percent survivals and in sizes of larvae was too great to show effects of rotifer concentrations or stock densities on those responses. It was not possible to conclude that total yield differed among the 25 to 1000 per liter rotifer concentrations, but yield at the 5000 per liter concentration did not overlap those at other concentrations.

There was no indication from inspection of the .90 confidence limits that the range of variability in responses was greater for the rotifer diet than for the zooplankton diet (Table 7). But, the relative variability, based upon inspection of standard errors and calculated coefficients of variation ($s/\bar{x}$) tended to be higher for rotifer-fed seatrout larvae, particularly for the growth and yield responses.

Lined Sole

Some of the data from stock density - food concentration experiments already have been included in published papers on lined soles and other species (Houde 1977, 1978). Only information on the zooplankton food was included in those reports. A summary of results for zooplankton and rotifers is given here (Table 8).

Survival ranged from 0.0 to 77.8% over all factor combinations for larvae fed zooplankton and from 0.0 to 55.6% for larvae fed rotifers. Survival increased significantly as food levels were raised for both zooplankton and rotifer foods (Tables 9 and 10). Survival decreased significantly and steadily as stock density increased (Tables 9 and 10) but only the survival rate at the lowest stock density, 0.5 per liter, could be shown to differ significantly from rates at other stock densities for both zooplankton and rotifers. For all factor combinations, mean percent survivals were nearly identical for zooplankton and rotifers, 19.1 and 19.7% respectively. There was some evidence that survival was higher on rotifers at the lowest food levels and on zooplankton at the highest food level (Table 9). At food levels of 1000 per liter or higher, either zooplankton or rotifers could be used to rear large numbers of lined soles to metamorphosis.

Lined soles grew relatively fast on zooplankton compared to rotifers. Over all factor combinations, mean standard lengths at 16 days were 3.93 mm on the zooplankton diet and only 3.40 mm on the rotifer diet. The difference in mean dry weights at 16 days was even more striking; survivors averaged 209 µg on the zooplankton diet but only 123-µg on the rotifer diet. For both diets, growth increased significantly as food level was raised (Tables 9 and 10). Mean standard lengths and dry weights were significantly higher at the 1000 per liter food levels compared to the 50 and 100 per liter levels for both diets. For lined sole, condition factors (defined as larval dry weight divided by standard length cubed) were calculated. The mean condition factors over all factor combinations were 2.90 for zooplankton and 2.67 for rotifers. Although larvae reared on zooplankton may have been in better

TABLE 7. SPOTTED SEATROUT. NINETY PERCENT CONFIDENCE INTERVALS ABOUT THE MEANS FOR PERCENT SURVIVAL, STANDARD LENGTH, DRY WEIGHT PER LARVA AND TOTAL YIELDS OF LARVAE AT 12 DAYS AFTER HATCHING REARED AT 28°C ON EITHER A ZOOPLANKTON OR ROTIFER DIET. CONFIDENCE LIMITS ARE PLACED ON MEANS OVER ALL FACTOR COMBINATIONS AT THE DESIGNATED FACTOR LEVEL.

Factor and level	.90 CONFIDENCE LIMITS			
	Percent survival	Standard length (mm)	Larval dry weight (µg)	Total yield (mg)
I. Zooplankton Food 28°				
Food Concentration				
25	6.7 to 15.1	3.13 to 4.75	30.7 to 341.7	0.0 to 5.2
100	18.7 to 33.6	2.71 to 5.85	0.0 to 509.7	6.2 to 11.1
1000	49.3 to 53.1	4.31 to 7.51	0.0 to 1285.4	65.6 to 74.0
5000	65.7 to 73.4	5.75 to 8.81	205.7 to 2214.0	166.2 to 179.2
Stock Density				
0.5	44.5 to 51.7	7.23 to 10.37	1159.6 to 3067.4	5.3 to 12.5
5.0	37.2 to 52.0	5.52 to 8.48	697.9 to 1628.7	25.5 to 33.1
25.0	15.4 to 29.4	5.18 to 6.88	453.0 to 938.2	33.3 to 41.7
II. Rotifer Food 28°				
Food Concentration				
25	10.9 to 35.5	2.33 to 3.57	0.0 to 119.7	0.0 to 8.9
100	36.3 to 44.3	2.68 to 4.38	0.0 to 233.1	2.0 to 10.0
1000	31.8 to 53.6	3.42 to 4.10	77.2 to 122.8	0.0 to 13.1
5000	56.9 to 80.5	3.50 to 4.42	44.1 to 224.7	15.2 to 22.0
Stock Density				
0.5	21.3 to 49.7	3.88 to 5.20	87.3 to 372.1	0.0 to 6.9
5.0	47.9 to 53.3	3.78 to 4.50	96.1 to 197.7	7.0 to 12.4
25.0	39.9 to 50.1	3.26 to 3.86	65.1 to 115.1	18.2 to 24.6

TABLE 8. LINED SOLE. THE EFFECTS OF STOCKING DENSITY AND FOOD CONCENTRATION ON SURVIVAL AND GROWTH OF LARVAE AT 16 DAYS AFTER HATCHING AND 28°C. A ZOOPLANKTON AND ROTIFER DIET ARE COMPARED.

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
I. Zooplankton Food							
0.5	50	48	0	0.0	-	-	0.0
0.5	50	58	1	5.6	3.32	70.0	0.1
0.5	100	104	5	27.8	3.71	142.0	0.7
0.5	100	184	2	11.1	3.98	197.5	0.4
0.5	1000	1023	14	77.8	4.74	392.9	5.5
0.5	1000	910	12	66.7	4.59	376.7	4.5
2.0	50	68	0	0.0	-	-	0.0
2.0	50	59	0	0.0	-	-	0.0
2.0	100	107	7	10.0	3.67	132.9	0.9
2.0	100	98	3	4.3	2.99	30.0	0.1
2.0	1000	1032	41	58.6	4.09	252.7	10.4
2.0	1000	1006	10	14.3	4.54	152.0	1.5
8.0	50	63	0	0.0	-	-	0.0
8.0	50	49	20	7.1	3.31	84.0	1.7
8.0	100	102	13	4.6	3.33	114.6	1.5
8.0	100	90	13	4.6	3.65	137.7	1.8
8.0	1000	1077	157	56.1	4.35	255.9	40.2
8.0	1000	962	127	45.4	4.11	214.1	27.2
16.0	50	87	0	0.0	-	-	0.0
16.0	50	57	0	0.0	-	-	0.0
16.0	100	119	18	3.2	3.54	116.7	2.1
16.0	100	127	0	0.0	-	-	0.0
16.0	1000	906	86	15.4	3.78	207.0	17.8
16.0	1000	961	258	46.1	3.67	185.9	48.0

(continued)

TABLE 8 (CONTINUED)

Stock density (no. liter^{-1})	Nominal food concentration (no. liter^{-1})	Mean food concentration (no. liter^{-1})	Survival		Mean standard length (mm)	Mean larva dry weight (µg)	Total yield dry weight (mg)
			number	percent			
II. Rotifer Food							
0.5	50	79	3	16.7	3.40	90.0	0.3
0.5	50	61	4	22.2	3.33	70.0	0.3
0.5	100	203	5	27.8	3.30	112.0	0.6
0.5	100	142	2	11.1	3.38	52.5	0.1
0.5	1000	1626	10	55.6	3.56	177.0	1.8
0.5	1000	1286	13	72.2	3.95	148.5	1.9
2.0	50	102	0	0.0	-	-	0.0
2.0	50	146	6	8.6	3.15	53.3	0.3
2.0	100	120	27	38.6	3.54	132.6	3.6
2.0	100	93	9	12.9	3.20	77.8	0.7
2.0	1000	1292	15	21.4	3.52	162.7	2.4
2.0	1000	956	20	28.6	3.90	196.0	3.9
8.0	50	72	6	2.1	3.09	33.3	0.2
8.0	50	62	15	5.4	3.34	124.0	1.9
8.0	100	144	38	13.6	3.17	70.5	2.7
8.0	100	329	22	7.9	3.29	60.9	1.3
8.0	1000	1409	64	22.9	3.42	124.0	7.9
8.0	1000	1378	78	27.9	3.83	156.4	12.2
16.0	50	88	2	0.4	3.18	98.5	0.2
16.0	50	81	1	0.2	2.80	46.0	0.1
16.0	100	126	50	8.9	2.94	68.4	3.4
16.0	100	166	88	15.7	3.64	149.1	13.1
16.0	1000	887	115	20.5	3.14	110.4	12.7
16.0	1000	979	170	30.4	3.37	130.8	22.2

TABLE 9. LINED SOLE. MEAN PERCENT SURVIVALS, LENGTHS, DRY WEIGHTS AND TOTAL YIELDS OF LARVAE REARED ON ZOOPLANKTON AND ON ROTIFER DIETS AS FUNCTIONS OF FOOD CONCENTRATION AND STOCK DENSITY. THE VALUES AND STANDARD ERRORS ARE MEANS OVER ALL FACTOR COMBINATIONS FOR EACH FACTOR LEVEL.

	Percent survival		Standard length (mm)		Dry weight per larva (µg)		Total yield (mg)	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
I. Zooplankton								
Food Concentration								
50	1.6	1.0	3.31	0.01	77.0	7.0	0.2	0.2
100	8.2	3.1	3.55	0.12	124.5	18.9	0.9	0.3
1000	47. 5	8.0	4.23	0.14	254.7	30.8	19.4	6.2
Stock Density								
0.5	31.5	13.5	4.06	0.26	235.8	64.2	1.9	1.0
2.0	14.5	9.1	3.82	0.33	141.9	45.6	2.2	1.7
8.0	19.6	10.0	3.75	0.21	161.3	32.0	12.1	7.0
16.0	10.8	7.5	3.66	0.07	169.9	27.3	11.3	7.9
II. Rotifers								
Food Concentration								
50	6.9	3.0	3.18	0.08	64.4	12.2	0.4	0.2
100	17.1	3.8	3.31	0.08	90.5	12.7	3.2	1.5
1000	34.9	6.6	3.59	0.10	150.7	10.0	8.1	2.6
Stock Density								
0.5	34.3	9.9	3.49	0.10	108.3	19.4	0.8	0.3
2.0	18.3	5.7	3.46	0.14	103.7	26.4	1.8	0.7
8.0	13.6	4.2	3.36	0.11	94.9	19.2	4.3	1.9
16.0	12.7	4.8	3.18	0.12	100.5	15.7	8.6	3.6

TABLE 10. LINED SOLE. RESULTS OF ANALYSES OF VARIANCE EXAMINING EFFECTS OF FOOD CONCENTRATION AND STOCK DENSITY ON LARVAE REARED ON EITHER A ZOOPLANKTON OR ROTIFER DIET AT 28°C. DUPLICATED EXPERIMENTS WERE RUN AT FACTOR COMBINATIONS OF 50, 100 AND 1000 PREY LITER^{-1} AND 0.5, 2.0, 8.0 AND 16.0 LITER^{-1} STOCK DENSITY.

	Source of variation	Degrees of freedom	Mean percent survival	Overall mean standard length	Overall mean dry weight per larva	Mean total dry weight yield
I.	Zooplankton					
	Food concentration, P	2	40.86**	10.09**	8.96**	21.14**
	Stock density, S	3	4.13**	0.45	0.77	1.40
	P, S	6	0.52	-	-	0.44
	Error	12				
II.	Rotifers					
	Food concentration, P	2	26.39**	5.52*	12.25**	14.17**
	Stock density, S	3	8.22**	1.49	0.15	2.37
	P, S	6	1.87	-	1.26	
	Error	12				

* Significant at the .05 level

** Significant at the .01 level

condition, it also is possible that larger mean sizes of zooplankton-reared larvae combined with allometric growth could have caused the difference in condition factor. In that case, a higher condition factor would simply reflect more advanced development and not better condition of the larvae.

Total dry weight yields at the end of experiments differed greatly between the two diets. Over all factor combinations, mean total yield was 6.8 mg for zooplankton but only 3.9 mg for rotifers. Significant increases in total yield were observed at each increasing food level for both diets (Tables 8 and 9). Total yield for the zooplankton diet was nearly 100 times higher at 1000 per liter food level than at 50 per liter, while for the rotifer diet total yield was 20 times higher at 1000 per liter than at 50 per liter. Surprisingly, the analyses of variance did not detect significant differences in total yield attributable to stock densities (Tables 9 and 10). It did seem that total yields were much higher at the 8.0 and 16.0 per liter stock levels than at 0.5 and 2.0 levels (Table 9). For the zooplankton diet, total yield averaged 5.7 times higher at the two highest stock densitites compared to the two lowest densities. For the rotifer diet, total yield at the two highest stock levels averaged 5.0 times higher than yields at the two lowest stock levels.

Predictability: The .90 confidence limits on percent survival, mean standard length, mean dry weight per survivor and total yield indicated that effects of food concentration can be predicted with reasonable confidence (Table 11). For both zooplankton and rotifers the .90 confidence limits on mean estimates at 1000 per liter food level either did not overlap those at 100 per liter or overlapped only slightly. The variability was great enough that differential responses between the 50 and 100 per liter food levels could not be detected with any degree of confidence. The precision with which estimates could be made generally increased as food level increased. The relatively uncertain results of the analyses of variance with regard to differences among stock levels, combined with high variance estimates at all levels of the factors that were tested, left little hope that stock density effects could be detected with confidence. Nevertheless, it was apparent that lined sole survival rates and growth rates tended to decrease at high stock levels. If it is important to maximize survival rate or larval size in an experiment, as opposed to total yield, then low stocking rates should be used.

For lined sole there was no indication that the rotifer diet gave more variable results than the zooplankton diet (Table 11). In fact, when coefficients of variation ($s/\bar{x}$) were compared, they tended to be slightly lower for rotifers. At a moderately high food level of 1000 per liter either zooplankton or rotifers could be used in a standard culture procedure for lined sole.

ROTIFER-*ARTEMIA* VS. ZOOPLANKTON DIETS

Either laboratory-cultured foods (rotifers-*Artemia*) or net-collected zooplankton when fed at levels of 500 to 1000 per liter in a prescribed regime (see Experimental Methods) can be used to rear larvae from hatching to well beyond metamorphosis. Survival rates were nearly identical between the

TABLE 11. LINED SOLE. NINETY PERCENT CONFIDENCE INTERVALS ABOUT THE MEANS FOR PERCENT SURVIVAL, STANDARD LENGTH, DRY WEIGHT PER LARVA AND TOTAL YIELDS OF LINED SOLE AT 16 DAYS AFTER HATCHING REARED AT 28°C ON EITHER A ZOOPLANKTON OR ROTIFER DIET. CONFIDENCE LIMITS ARE PLACED ON MEANS OVER ALL FACTOR COMBINATIONS AT THE DESIGNATED FACTOR LEVEL.

	.90 CONFIDENCE LIMITS			
	Percent survival	Standard length (mm)	Larval dry weight (µg)	Total yield (mg)
I. Zooplankton Food				
28°				
Food Concentration				
50	0.0 to 2.0	3.31 to 3.33	56.6 to 97.4	0.0 to <0.1
100	2.2 to 12.5	3.32 to 3.78	87.8 to 161.0	<0.1 to 1.8
1000	30.8 to 63.2	3.97 to 4.49	196.3 to 313.1	5.3 to 26.2
Stock Density				
0.5	2.6 to 60.8	3.50 to 4.62	99.1 to 372.6	<0.1 to 4.6
2.0	0.0 to 30.0	3.12 to 4.52	34.5 to 249.3	<0.1 to 3.1
8.0	0.8 to 38.8	3.31 to 4.19	93.1 to 229.5	<0.1 to 35.9
16.0	0.0 to 21.7	3.51 to 3.81	90.2 to 249.5	0.1 to 10.6
II. Rotifer Food				
28°				
Food Concentration				
50	0.8 to 10.7	3.04 to 3.32	37.9 to 90.9	<0.1 to 0.6
100	10.1 to 23.1	3.16 to 3.46	66.4 to 114.6	0.6 to 4.0
1000	22.5 to 47.3	3.40 to 3.78	131.7 to 169.8	2.9 to 10.5
Stock Density				
0.5	13.9 to 55.4	3.28 to 3.70	67.0 to 149.7	0.2 to 1.4
2.0	3.6 to 32.0	3.17 to 3.75	40.0 to 167.5	<0.1 to 6.9
8.0	4.5 to 21.9	3.13 to 3.59	54.0 to 135.7	0.6 to 7.9
16.0	1.4 to 23.3	2.92 to 3.44	67.1 to 134.0	0.2 to 20.3

two diets for both spotted seatrout and lined soles, but growth differed.

Spotted Seatrout

At 16 days after hatching percent survivals ranged from 20.0 to 65.7% for experiments in which egg source and diet were compared (Table 12). A two-way analysis of variance did not detect any significant differences ($P >> .50$) among survival rates attributable to egg source or diet. Mean percent survival was approximately 45% for larvae reared from planktonic eggs or from eggs produced by hormone-induced spawning. Survival also averaged 45% for larvae fed on zooplankton or on rotifers-Artemia.

Growth of seatrout larvae did differ significantly ($P < .025$) between the two types of diet, but not between egg sources ($P > .25$). Mean length of zooplankton-fed larvae was 14.38 mm, while that of rotifer-Artemia-fed larvae was 9.30 mm, a difference of more than 5.0 mm (Table 12).

Seatrout larvae appeared to be healthy on both diets but, as previously reported for the stock density-food concentration experiments, better growth can be achieved on zooplankton than on the cultured rotifers and Artemia. In this series of experiments it was demonstrated that the growth advantage attributable to zooplankton continued past metamorphosis, although we did not investigate the reason for the observed differences in growth between diets. We attempted to make food of both types available in approximately equal dry weight amounts. Still, we do not know whether the growth differences due to diet were caused by nutritional differences in the diets or if they were related to size-selective predation by the seatrout larvae on the relatively variable zooplankton prey.

Lined Sole

At 25 days after hatching percent survivals of zooplankton and rotifer-Artemia-fed lined soles ranged from 40.0 to 75.7% (Table 13). Mean percent survivals for the two diets over three replicates were identical, 56.7% and the variability in observed survival was similar between the diets. Either diet is suitable to rear large numbers of apparently healthy, metamorphosed lined soles.

Standard lengths were longer for rotifer-Artemia-fed individuals than for zooplankton-fed individuals (Table 13), but a t-test did not detect significant differences ($.05 < P < .10$) in the mean lengths of 7.19 and 6.48 mm. Variability in lengths was similar between diets, the coefficients of variation ranging from 8-13% for zooplankton and 7-11% for rotifers-Artemia.

Survival of lined soles well after metamorphosis was independent of diet type just as larval survival was independent of the rotifer or zooplankton diet in the stock density-food concentration experiments. The growth response after metamorphosis differed from that observed in the larval experiments. Although lined sole larvae were not measured on day 13 (the day that Artemia nauplii were first added to the rotifer diet), it was obvious that zooplankton-fed larvae were larger on that day, as was observed

TABLE 12. SPOTTED SEATROUT. PERCENT SURVIVAL AND MEAN LENGTH OF LARVAE REARED TO 16 DAYS AFTER HATCHING AT 28°C ON EITHER A ROTIFER-ARTEMIA OR ZOOPLANKTON DIET. TWO EGG SOURCES, PLANKTON-COLLECTED OR HORMONE-INDUCED, ALSO WERE COMPARED.

Egg source	Food type	Percent survival	Mean standard length (mm) (±1 S.E.)
Plankton	Rotifer-Artemia	65.7	11.97 ± 0.19
Plankton	Rotifer-Artemia	27.1	8.56 ± 0.18
Hormone	Rotifer-Artemia	20.0	8.94 ± 0.34
Hormone	Rotifer-Artemia	62.9	7.72 ± 0.09
Plankton	Zooplankton	60.0	15.49 ± 0.22
Plankton	Zooplankton	21.4	14.82 ± 0.33
Hormone	Zooplankton	37.1	15.51 ± 0.17
Hormone	Zooplankton	64.3	11.69 ± 0.11

Means

Percent Survival

Plankton eggs	43.6%
Hormone eggs	46.1%
Rotifer-Artemia diet	43.9%
Zooplankton diet	45.7%

Standard Length

Plankton eggs	12.71mm
Hormone eggs	10.97mm
Rotifer-Artemia diet	9.30mm
Zooplankton diet	14.38mm

TABLE 13. LINED SOLE. PERCENT SURVIVAL AND MEAN LENGTH OF LINED SOLES REARED TO 25 DAYS AFTER HATCHING AT 28°C ON EITHER A ROTIFER-ARTEMIA OR ZOOPLANKTON DIET.

Food type	Percent survival	Mean standard length (mm) (±1 S.E.)
Rotifer-Artemia	54.3	7.72 ± 0.11
Rotifer-Artemia	75.7	7.01 ± 0.07
Rotifer-Artemia	40.0	6.84 ± 0.15
Zooplankton	51.4	6.77 ± 0.14
Zooplankton	48.6	6.29 ± 0.11
Zooplankton	70.0	6.38 ± 0.08

Means

Percent Survival	
Rotifer-Artemia	56.7%
Zooplankton	56.7%
Standard Length	
Rotifer-Artemia	7.19mm
Zooplankton	6.48mm

previously in the stock density-food concentration experiments. After addition of Artemia nauplii, lined sole growth rate on the rotifer-Artemia diet increased greatly. We believe that the differences in size of lined soles reared on the two diets would have been significant if the experiment had continued a few more days. We do not know if the increased growth rate attributable to Artemia nauplii had a nutritional basis or whether Artemia simply were relatively easy prey to capture compared to more agile copepods by the bottom dwelling, lined sole juveniles.

ARTEMIA NAUPLII VS. ZOOPLANKTON DIET FOR JUVENILE LINED SOLE

For lined sole that had been fed zooplankton at 1000 per liter until they were near metamorphosis (day 13) and subsequently tested on Artemia nauplii or > 280 µm zooplankton diets, there was little difference in growth between days 13 and 25 (Table 14). Nested analysis of variance carried out on days 17, 21 and 25 detected no significant differences in mean lengths ($P > .50$) on any of the days, but on each day the differences among replicates were judged significant ($P < .001$), indicating that among replicate variability in growth was much greater than that due to diets. Survival of juvenile lined soles used in these experiments was near 100% for both diets.

Based on the wet weight data, specific growth rates were estimated from the fitted exponential growth curves. The growth equation for 13-25 day-old lined sole fed on Artemia was $W_t = 0.2032e^{0.1887t}$ and that for the larvae fed on > 280 µm zooplankton was $W_t = 0.2519e^{0.1723t}$, where

W_t = wet weight (mg) at age t

t = age in days.

The specific growth rates were 20.8% per day for lined soles fed Artemia and 18.8% per day for lined soles fed zooplankton. The instantaneous growth coefficients in the two equations ($g = 0.1887$ and $g = 0.1723$) did not differ significantly (t-test; $P > .50$).

If the experiments had continued beyond day 25 it is possible that growth on Artemia would have exceeded that on zooplankton. Mean lengths and weights of the Artemia-fed individuals were beginning to diverge from those of zooplankton-fed fish on days 21 and 25 (Table 14). Young juvenile lined soles can be reared routinely on Artemia nauplii with survival and growth rates at least as good as those derived from a zooplankton diet.

GROWTH RATES, FEEDING RATES AND RATIONS

Food consumption rates of both spotted seatrout and lined sole larvae were estimated from the time that they began to feed until they were metamorphosing. For both species there was an increase in feeding rate that was related to age and size of larvae. Feeding rates also increased as food concentrations were raised. Taniguchi (1979) gave a preliminary analysis of feeding rate and growth rate information for spotted seatrout. Houde and Schekter (1979, in press a, in press b) have reported on lined sole feeding

TABLE 14. LINED SOLE. MEAN STANDARD LENGTHS AND WET WEIGHTS OF JUVENILES FED ON ARTEMIA NAUPLII OR WILD PLANKTON (>280 µm width). ALL LARVAE WERE FED ON WILD PLANKTON AT 1000 PER LITER CONCENTRATION FROM DAY 3 TO DAY 13. AT DAY 13, HALF OF THE POPULATION WAS TRANSFERRED TO THE ARTEMIA DIET AND HALF TO THE >280 µm ZOOPLANKTON DIET. THREE REPLICATES OF THE EXPERIMENT WERE RUN. IN TWO REPLICATES, EXPERIMENTS WERE RUN UNTIL DAY 25, BUT THE THIRD WAS TERMINATED AT DAY 21. MEAN LENGTHS AND WEIGHTS ARE FROM 10 SPECIMENS.

Days after hatching	Diet	Mean standard length (mm)	Mean wet weight (mg)
13	Zooplankton	3.49	2
		3.92	2
		4.21	3
		$\bar{x}$. = 3.87	$\bar{x}$. = 2.3
17	Artemia	5.13	4
		5.61	5
		5.58	6
		$\bar{x}$. = 5.44	$\bar{x}$. = 5.0
	>280 µm Zooplankton	5.16	4
		5.62	5
		5.98	6
		$\bar{x}$. = 5.59	$\bar{x}$. = 5.0
21	Artemia	6.33	11
		6.74	11
		8.00	15
		$\bar{x}$. = 7.02	$\bar{x}$. = 12.3
	>280 µm Zooplankton	6.06	5
		7.33	12
		7.73	14
		$\bar{x}$. = 7.04	$\bar{x}$. = 10.3

(continued)

TABLE 14 (CONTINUED)

Days after hatching	Diet	Mean standard length (mm)	Mean wet weight (mg)
25	Artemia	8.47	15
		9.60	27
		$\bar{x}$. = 9.04	$\bar{x}$. = 21.0
	>280 µm Zooplankton	8.72	15
		8.97	22
		$\bar{x}$ = 8.85	$\bar{x}$. = 18.5

rates and growth rates as parts of more inclusive studies that also considered other species.

Spotted Seatrout

Spotted seatrout larvae grew fast when temperatures were near optimum and food concentrations were adequate. Equations that describe growth in length and weight of larvae that were fed zooplankton or rotifer diets are given in Table 15. At 28°C, growth rates were significantly higher on the zooplankton diet than on the rotifer diet. On zooplankton food, seatrout larvae generally increased their rate of growth as temperature increased from 24 to 32°C, except at the lowest food level that was tested.

Instantaneous growth coefficients in the exponential equations describing growth in weight ranged from 0.1616 to 0.5683 over all prey concentrations and temperatures that were tested (Table 15). Those coefficients correspond to specific growth rates of 17.5 to 76.5% per day. Growth coefficients for any of the food concentrations always were higher for zooplankton-fed larvae than for rotifer-fed larvae. The difference in growth rates between diets was most pronounced at the 1000 per liter food level. The significance of the differences in growth between diets is easily seen in Table 16, where predicted lengths and weights can be compared. At 12 days after hatching and 28°C, zooplankton-fed seatrout were 1.8 times heavier than rotifer-fed seatrout at 25 per liter food level, 1.5 times heavier at 100 per liter food level and 6.0 times as heavy at the 1000 per liter food level.

Although seatrout larvae were capable of fast growth at all temperatures from 24 to 32°C, growth was significantly faster at 28 and 32° than at 24°C. The growth rate at 25 per liter food level did decrease as temperature was raised from 28 to 32°C, indicating that increased metabolic demands at high temperatures may reduce the potential for larval growth at that low food concentration. Although growth rates at the 100 and 1000 per liter food levels were high at 32°C, there was a strong tendency for seatrout larvae to be cannibalistic at that high temperature. Larvae were noticeably irritable and aggressive at 32°C. Cannibalism and attempted cannibalism were believed to be significant sources of mortality for 32°C larvae. The fast growth and relatively high survival that were obtained at 28°C indicated that this temperature, combined with food levels of 100 to 1000 per liter, will provide better conditions for seatrout larvae culture than higher or lower temperatures.

Spotted seatrout larvae were capable of consuming zooplankton or rotifers at high rates, especially when temperatures were 28°C or higher. Under those conditions and 1000 per liter food levels larvae consumed 200-800% of their body weights per day, during the first six days after hatching (Table 16). Spotted seatrout larvae feeding rates are extraordinary and have not been matched by any other species that we have tested to date. At all food levels, seatrout larvae consumed rotifers at a faster rate than zooplankton until six days after hatching (Table 16). Slow-swimming rotifers apparently were relatively easy prey for small larvae to capture. As larvae grew their predatory ability increased. By six days after hatching they fed at a higher rate on zooplankton than on rotifers. The relatively

TABLE 15. SPOTTED SEATROUT. EQUATIONS DESCRIBING GROWTH OF LARVAE REARED ON EITHER ZOOPLANKTON OR ROTIFER DIETS. EQUATIONS ARE GIVEN FOR 24, 28 and 32°C FOR THE ZOOPLANKTON DIET BUT ONLY FOR 28°C FOR THE ROTIFER DIET. THE RELATIONSHIPS ARE GIVEN FOR THREE FOOD CONCENTRATIONS AT EACH OF THE FOOD TYPE AND TEMPERATURE CONDITIONS.

Food concentration	Days after hatching	Variable	Regression equation	Number of observations	Standard error of regression coefficient	Coefficient of determination
I. Zooplankton Diet						
A. 24°C						
25/1	2-12	Growth in length (mm)	$L = 1.6540 \exp (0.0453t)$	185	0.0032	.53
	2-12	Growth in weight (µg)	$W_t = 7.1128 \exp (0.1616t)$	13	0.0209	.85
100/1	2-12	Growth in length (mm)	$L = 1.7506 \exp (0.0729t)$	177	0.0022	.87
	2-12	Growth in weight (µg)	$W_t = 4.2749 \exp (0.2984t)$	14	0.0187	.96
1000/1	2-12	Growth in length (mm)	$L = 1.6593 \exp (0.1173t)$	133	0.0018	.97
	2-12	Growth in weight (µg)	$W_t = 3.4515 \exp (0.4687t)$	12	0.0078	1.00
B. 28°C						
25/1	2-12	Growth in length (mm)	$L = 1.8547 \exp (0.0890t)$	141	0.0025	.90
	2-12	Growth in weight (µg)	$W_t = 4.9762 \exp (0.3706t)$	12	0.0134	.99
100/1	2-12	Growth in length (mm)	$L = 1.9477 \exp (0.0879t)$	130	0.0025	.91
	2-12	Growth in weight (µg)	$W_t = 5.9567 \exp (0.3603t)$	12	0.0316	.93
1000/1	2-12	Growth in length (mm)	$L = 1.8259 \exp (0.1434t)$	134	0.0013	.99
	2-12	Growth in weight (µg)	$W_t = 6.4701 \exp (0.5269t)$	12	0.0210	.98

(continued)

TABLE 15 (CONTINUED)

Food concentration	Days after hatching	Variable	Regression equation	Number of observations	Standard error of regression coefficient	Coefficient of determination
C. 32°C						
25/1	2-12	Growth in length (mm)	$L = 1.8473 \exp(0.0620t)$	74	0.0027	.88
	2-12	Growth in weight (µg)	$W_t = 6.3810 \exp(0.2410t)$	7	0.0343	.91
100/1	2-12	Growth in length (mm)	$L = 1.7701 \exp(0.1123t)$	57	0.0024	.97
	2-12	Growth in weight (µg)	$W_t = 6.2125 \exp(0.4241t)$	6	0.0203	.99
1000/1	2-12	Growth in length (mm)	$L = 1.7215 \exp(0.1666t)$	55	0.0025	.99
	2-12	Growth in weight (µg)	$W_t = 7.8044 \exp(0.5683t)$	6	0.0499	.97
II. Rotifer Diet						
A. 28°C						
25/1	2-12	Growth in length (mm)	$L = 1.9317 \exp(0.0764t)$	119	0.0022	.91
	2-12	Growth in weight (µg)	$W_t = 6.7756 \exp(0.2964t)$	10	0.0198	.97
100/1	2-12	Growth in length (mm)	$L = 2.0156 \exp(0.0776t)$	96	0.0020	.94
	2-12	Growth in weight (µg)	$W_t = 7.6457 \exp(0.3074t)$	8	0.0244	.96
1000/1	2-12	Growth in length (mm)	$L = 1.9451 \exp(0.0972t)$	83	0.0027	.94
	2-12	Growth in weight (µg)	$W_t = 9.3176 \exp(0.3472t)$	7	0.0440	.93

TABLE 16. SPOTTED SEATROUT. PREDICTED SIZES, FEEDING RATES, RATIONS AND WEIGHT SPECIFIC RATIONS FOR LARVAE AT SELECTED AGES. LENGTHS AND WEIGHTS OF LARVAE ARE PREDICTED FROM EQUATIONS IN TABLE 15. FEEDING RATES AND RATIONS ARE THE MEAN VALUES BASED ON THREE REPLICATE EXPERIMENTS FOR LARVAE AT EACH AGE, TEMPERATURE AND DIET.

Food concentration	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.larva)
I. Zooplankton Diet						
A. 24°C						
25/1	2	1.8	9.8	0.02	0.44	0.05
	4	2.0	13.6	0.06	1.26	0.10
	6	2.2	18.8	0.12	2.87	0.15
	8	2.4	25.9	0.22	5.33	0.21
	10	2.6	35.8	0.39	9.36	0.26
	12	2.8	49.5	0.67	15.96	0.32
100/1	2	2.0	7.8	0.05	1.23	0.16
	4	2.3	14.1	0.14	3.46	0.25
	6	2.7	25.6	0.33	7.90	0.31
	8	3.1	46.5	0.70	16.76	0.36
	10	3.6	84.5	1.44	34.46	0.41
	12	4.2	153.5	2.91	69.77	0.45
1000/1	2	2.1	8.8	0.35	8.34	0.95
	4	2.7	22.5	0.81	19.35	0.86
	6	3.4	57.5	1.80	43.31	0.75
	8	4.2	146.7	3.98	95.48	0.65
	10	5.4	374.6	8.71	209.09	0.56
	12	6.8	956.4	19.02	456.49	0.48

(continued)

TABLE 16 (CONTINUED)

Food concentration	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.larva)
B. 28°C						
25/1	2	2.2	10.4	0.03	0.74	0.07
	4	2.6	21.9	0.17	3.99	0.18
	6	3.2	46.0	0.55	13.31	0.29
	8	3.8	96.5	1.67	40.07	0.42
	10	4.5	202.5	4.87	116.82	0.58
	12	5.4	424.9	14.04	337.03	0.79
100/1	2	2.3	12.2	0.23	5.63	0.46
	4	2.8	25.2	0.62	14.82	0.59
	6	3.3	51.7	1.53	36.76	0.71
	8	3.9	106.4	3.71	89.10	0.84
	10	4.7	218.7	8.92	214.00	0.98
	12	5.6	449.5	21.33	512.02	1.14
1000/1	2	2.4	18.6	3.10	74.31	4.00
	4	3.2	53.2	5.49	131.73	2.47
	6	4.3	152.7	9.71	232.93	1.53
	8	5.8	438.1	17.14	411.28	0.94
	10	7.7	1256.6	30.23	725.60	0.58
	12	10.2	3604.7	53.32	1279.57	0.36

(continued)

TABLE 16 (CONTINUED)

Food concentration	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.larva)
C. 32°C						
25/1	2	2.1	10.3	0.28	6.72	0.65
	4	2.4	16.7	0.54	13.06	0.78
	6	2.7	27.1	1.03	24.61	0.91
	8	3.0	43.9	1.90	45.64	1.04
	10	3.4	71.0	3.50	83.95	1.18
	12	3.9	115.0	6.40	153.71	1.34
100/1	2	2.2	14.5	1.01	24.13	1.66
	4	2.8	33.9	2.09	50.06	1.48
	6	3.5	79.1	4.28	102.74	1.30
	8	4.3	184.8	8.74	209.77	1.13
	10	5.4	431.6	17.80	427.23	0.99
	12	6.8	1008.0	36.21	869.05	0.86
1000/1	2	2.4	24.3	4.51	108.34	4.46
	4	3.4	75.8	8.46	202.99	2.68
	6	4.7	236.2	15.81	379.55	1.61
	8	6.5	735.9	29.54	708.93	0.96
	10	9.1	2293.2	55.14	1323.42	0.58
	12	12.7	7145.9	102.91	2469.78	0.35

(continued)

TABLE 16 (CONTINUED)

Food concentration	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.larva)
II. Rotifer Diet						
28°C						
25/1	2	2.3	12.3	0.32	7.59	0.62
	4	2.6	22.2	0.46	10.94	0.49
	6	3.1	40.1	0.65	15.61	0.39
	8	3.6	72.6	0.92	22.09	0.30
	10	4.1	131.3	1.30	31.10	0.24
	12	4.8	237.5	1.82	43.64	0.18
100/1	2	2.4	14.1	1.29	31.05	2.20
	4	2.7	26.1	1.87	44.80	1.71
	6	3.2	48.4	2.69	64.45	1.33
	8	3.7	89.4	3.86	92.53	1.03
	10	4.4	165.4	5.53	132.66	0.80
	12	5.1	305.8	7.92	190.01	0.62
1000/1	2	2.4	18.7	6.36	152.64	8.18
	4	2.9	37.4	8.27	198.58	5.32
	6	3.5	74.8	10.76	258.27	3.45
	8	4.2	149.8	13.99	335.81	2.24
	10	5.1	300.0	18.19	436.53	1.46
	12	6.2	600.8	23.64	567.38	0.94

fast increase in feeding rate on zooplankton was accomplished by including successively larger stages of copepods in the diet as both larval mouth size and swimming ability increased.

There was a pronounced effect of temperature on feeding rates (Table 16). During the first six days after hatching seatrout larvae of equal age consumed zooplankton approximately five times faster at 28° than at 24°C and about 2.5 times faster at 32° than at 28°C. At 32°C, larvae consumed zooplankton about 11 times faster than at 24°C.

Lined Sole

Compared to spotted seatrout, lined sole grew slowly and had low feeding rates. Equations describing the growth and feeding rate relationships are given in Table 17. Growth rates and food consumption rates increased significantly when zooplankton concentration was raised from 100 to 1000 per liter.

The exponential model used to describe growth in weight gave instantaneous growth coefficients ranging from 0.1849 to 0.3259 (Table 17). These corresponded to specific growth rates of 20.3% per day at 50 per liter, 23.7% per day at 100 per liter and 38.5% per day at 1000 per liter food levels. Few larvae of lined sole can survive at zooplankton levels below 50 per liter; the 20% per day specific growth rate may be near the minimum rate at which lined sole larvae must grow to develop and survive.

Power functions described feeding rate ($\mu g\ hr^{-1}\ larva^{-1}$) of lined sole larvae as functions of age and of dry weight (Table 17). The feeding rate-weight function had the lower variance and it was used to estimate feeding rates at age, after lengths and weights were estimated from the Table 17 growth equations (Table 18).

Lined sole larvae consumed approximatley 200% of their body weights per day during the first week of feeding when food level was 1000 per liter but only 30-45% of their body weights per day at 100 per liter food level and about 15-20% of their body weights at the 50 per liter food level. During the first two days that larvae fed, when zooplankton mean dry weight was 0.15 µg per organism, larval feeding rates were 0.6, 1.5 and 11.2 food items per hr for 50, 100 and 1000 per liter zooplankton levels. The increase in feeding rates was approximately directly proportional to food concentration for 3-4 day-old larvae. The differences in feeding rates were less pronounced among older larvae but rates still were much higher at the 1000 per liter food level. The observed survival rates at metamorphosis for the three zooplankton concentrations were 1.4, 13.3 and 54.3% (Houde 1978). Survival could be increased beyond 54% if zooplankton levels were raised above 1000 per liter, but the increases in survival would be relatively small for the large increases in food level that would be necessary. For example, Houde's (1978) predictive equation indicated that a 2000 per liter zooplankton concentration would give 69% survival, a gain of 15% over that observed at 1000 per liter zooplankton concentration.

TABLE 17. LINED SOLE. EQUATIONS DESCRIBING GROWTH AND FEEDING RELATIONSHIPS FOR LARVAE REARED AT THREE FOOD CONCENTRATIONS.

Food concentration	Days after hatching	Variable	Regression equation	Number of observations	Standard error of regression coefficient	Coefficient of determination
50/1	4-17	Growth in length (mm)	$L = 1.712 + 0.105\ t$	26	0.009	.85
	4-17	Growth in weight (µg)	$W_t = 4.3239 \exp(0.1849t)$	23	0.0182	.83
	3-17	Feeding rate (µg hr^{-1})	$C = 0.0009\ t^{2.5230}$	9	0.9226	.52
			$C = 0.0060\ W^{1.1807}$	6	0.0730	.98
100/1	4-17	Growth in length (mm)	$L = 1.804 + 0.113\ t$	28	0.009	.85
	4-17	Growth in weight (µg)	$W_t = 4.8803 \exp(0.2129t)$	25	0.0190	.84
	3-17	Feeding rate (µg hr^{-1})	$C = 0.0125\ t^{1.8189}$	9	0.3117	.83
			$C = 0.0296\ W^{0.8555}$	7	0.0690	.97
1000/1	3-13	Growth in length (mm)	$L = 1.444 + 0.202\ t$	27	0.010	.94
	3-13	Growth in weight (µg)	$W_t = 3.6648 \exp(0.3259t)$	27	0.0147	.95
	3-13	Feeding rate (µg hr^{-1})	$C = 0.3242\ t^{1.2375}$	7	0.4218	.63
			$C = 0.4456\ W^{0.5418}$	7	0.0473	.96

L = standard length (mm)
W = dry weight (µg)
C = feeding rate (µg hr^{-1})
t = days after hatching

TABLE 18. LINED SOLE. PREDICTED SIZES, FEEDING RATES, RATIONS AND WEIGHT SPECIFIC RATIONS FOR LARVAE AT SELECTED AGES. LENGTHS AND WEIGHTS OF LARVAE ARE PREDICTED FROM EQUATIONS IN TABLE 17. FEEDING RATES ARE PREDICTED FROM THE FEEDING RATE-WEIGHT EQUATION IN TABLE 17. RATIONS ARE ESTIMATED FROM THE FEEDING RATE ESTIMATES THAT WERE ADJUSTED FOR FEEDING THAT OCCURRED AT NIGHT UNDER 50 LUX LIGHT LEVELS.

Food level	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.larva)
50/1	3	2.1	9.5	0.09	1.44	0.15
	4	2.1	8.7	0.08	1.28	0.15
	5	2.2	10.5	0.10	1.46	0.14
	7	2.5	15.2	0.15	3.14	0.21
	9	2.7	22.1	0.23	5.18	0.23
	11	2.9	32.0	0.36	7.65	0.24
	13	3.1	36.5	0.56	11.90	0.26
	15	3.3	67.5	0.87	18.49	0.27
	17	3.5	98.0	1.35	32.40	0.33
100/1	3	2.1	10.2	0.22	3.30	0.32
	4	2.3	11.4	0.24	3.59	0.31
	5	2.4	14.2	0.29	5.93	0.42
	7	2.6	21.7	0.41	9.63	0.44
	9	2.8	33.2	0.59	12.54	0.38
	11	3.0	50.8	0.85	18.07	0.36
	13	3.3	77.7	1.33	28.26	0.36
	15	3.5	119.0	1.77	42.48	0.36
	17	3.7	182.1	2.54	60.96	0.33

(continued)

TABLE 18 (CONTINUED)

Food level	Days after hatching	Standard length (mm)	Dry weight (µg)	Feeding rate (µg hr^{-1})	Ration (µg)	Weight specific ration (µg $µg^{-1}$.1arva)
1000/1	3	2.1	9.7	1.53	20.63	2.13
	4	2.3	13.5	1.83	24.67	1.83
	5	2.5	18.7	2.18	41.40	2.21
	7	2.9	35.9	3.10	67.63	1.88
	9	3.3	68.9	4.41	93.71	1.36
	11	3.7	132.2	6.28	150.72	1.14
	13	4.1	253.7	8.95	214.80	0.85

LENGTH-WEIGHT RELATIONSHIPS

Spotted Seatrout

The exponent in power functions that described the length-weight relationship for spotted seatrout larvae reared at three different temperatures, over three food levels (25, 100, 1000 food items per liter), differed significantly. The exponent at 28°C, for either rotifer or zooplankton diets, was significantly higher (Analysis of Covariance) than those at 24° or 32°C. The equations are:

$$\text{zooplankton food } 24^{\circ}\text{--}W = 1.07L^{3.41};$$

$$\text{zooplankton food } 28^{\circ}\text{--}W = 0.51L^{3.91};$$

$$\text{rotifer food } 28^{\circ}\text{--}W = 0.50L^{3.93};$$

$$\text{zooplankton food } 32^{\circ}\text{--}W = 0.83L^{3.62}.$$

There was no evidence that food concentrations had a significant effect on the length-weight relationships. The combined length-weight relationship for both of the food types, all temperature and food concentrations was: $W = 0.75L^{3.66}$.

Length-weight relationships, in theory, can be used as an index of larval condition. High values of the exponent in the power function relationships indicate that larvae are relatively "fat" and presumably better conditioned. We did not find effects of food concentration on length-weight relationships of spotted seatrout. This result indicates that the length-weight relationship may not be useful to judge effects of diet on larval condition for this species. Temperature did seem to have an easily detected effect on length-weight relationships for spotted seatrout. The "fattest" larvae, regardless of diet type, were reared at 28°C. Larvae at lower and higher temperatures apparently were less well conditioned.

Lined Sole

The only length-weight relationships that were obtained for lined sole were those at 28°C for both the zooplankton and rotifer diet, when food concentrations were 1000 per liter. There were no significant differences in the relationships:

$$\text{zooplankton food}\text{--}W = 0.60L^{4.03};$$

$$\text{rotifer food}\text{--}W = 0.63L^{4.05}.$$

Thus, there appeared to be no differences in condition of lined sole when reared at high food concentration for either type of food. The length-weight relationship from pooled zooplankton and rotifer-fed lined sole larvae was $W = 0.60L^{4.06}$.

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APPENDIX A

Procedures Used to Culture Larvae of Marine Fishes at the Rosenstiel School of Marine and Atmospheric Science

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Introduction

An ability to culture pelagic larvae of many marine fishes has been developed during the past several years at the Rosenstiel School. It is now possible to routinely conduct experimental research on laboratory-reared larvae or juveniles because culture methods allow production of larvae with predictable growth and survival rates. The method used in our work was adapted from that used by Mayo (1973) who reared many marine fishes during his graduate study at RSMAS. The basic techniques were improved upon over the years. Houde (1973) gave some procedures and discussed some problems of larval culture. Since that report there have been improvements in methods and we have gained a better understanding of larval culture requirements.

Our purpose here is to give details of methods we use to successfully culture tropical and temperate marine fishes from the embryo to the juvenile stage. Most of our emphasis is centered on techniques used to feed the larvae because many problems in larval culture are related to types, quantities and sizes of food organisms. Under optimum rearing conditions our method will predictably allow more than 50% of many species to survive from egg to juvenile, and for certain species up to 80% survival can be routinely obtained.

Obtaining Embryos

Pelagic eggs of many species are available during the four month, or longer, spawning seasons near Miami. The simplest way to obtain embryos of most species is to collect them in plankton nets. We use 1-m diameter plankton nets with either 333 µm or 505 µm meshes. Most eggs are retained by the 505 µm mesh, but a few species with small or elliptical eggs (e.g., Anchoa mitchilli) require use of the smaller mesh. For species that spawn in Biscayne Bay we set our collecting nets in the tidal flow from a dock at the laboratory. An egg collection usually consists of a 30-45 min net set. For reef or oceanic species surface tows of the plankton nets from a boat moving at about 1 knot are necessary. We use plankton nets to collect embryos of bay anchovy, lined soles and sea bream, species that are frequently used in our research.

Eggs are also obtained by hormone-induced spawning of adult females. Spotted seatrout eggs are obtained by injecting maturing females with human chorionic gonadotropin, using spawning techniques that have been described in the literature (Haydock, 1971; Kuo et al., 1974; Shehadeh and Ellis, 1970; Stevens, 1966, 1970). Biopsies of the ovary indicate if oocytes are maturing. We inject 1-2 IU HCG per gram body weight once or twice before the oocytes are ovulated, which occurs at 28 to 33 hrs after the first injection in spotted seatrout. Ovulated eggs are fertilized by the dry method. There is variability in egg quality from hormone-induced spawnings. Poor quality

can be recognized at the time eggs are fertilized. Multiple oil droplets, uncleared yolk material, and hypotonicity in eggs are obvious signs of poor quality.

Floating, fertilized pelagic eggs can be identified, sorted and counted by pipetting them into dishes of seawater adjusted to near-ambient temperatures. Only embryos at nearly the same stage of development are assigned to an experiment to insure that all hatched larvae are the same age. Because most spawning occurs at night, and eggs hatch about 15-30 hrs after fertilization for most fishes near Miami, there is usually only a single embryonic stage present in our plankton net egg collections. Glass pipettes of approximately 2 mm bore are used to select and count embryos before transferring them to incubation tanks, which usually are the rearing systems themselves. Prescribed experimental temperatures are obtained by adjusting incubation tank temperatures at a rate of $1^{o}C$ or less per hr.

Rearing Systems

Tanks

Culture tanks are 38 or 76-liter, all-glass rectangular aquaria. They are closed systems. Water is not filtered or recirculated during the experiments, although we routinely exchange from 10-20% of the tank volume on alternate days during the culture period. Open systems incorporating screened constant level siphons (Houde and Ramsay, 1971) or overflow standpipes can be used, but our best results have been obtained with the closed system, particularly during the first week after hatching. Tanks smaller than 38 liters give unpredictable survival of larvae; larger tanks sometimes give higher survival and better growth rates, but this reponse is species specific and can be evaluated. Tanks larger than 76 liters can be used if many larvae are required in an experiment, but large tank volumes require large numbers of food organisms to maintain nominal food concentrations. It often is more desirable to have replicated experiments in smaller tanks in which the food levels can be easily maintained.

Temperature

Temperature is controlled by 100-watt, immersion heaters and seldom varies by more than $\pm$ $0.5^{o}C$ from the designated experimental temperature. Temperature is checked and adjusted 2-3 times daily. Experimental temperatures usually range from $22-32^{o}C$ for the species that we rear.

Salinity

Salinity is maintained between 32 and $36^{o}/oo$, which is the usual range of salinities in Biscayne Bay, Florida. We have no evidence that salinity in that range is a critical factor for rearing larvae. Salinities are checked on alternate days during the culture period. If salinity reaches $36^{o}/oo$, tapwater is added in quantities that do not exceed 1.3% of the tank volume daily. That amount of tapwater reduces salinity by about $0.5^{o}/oo$ in the rearing tanks.

Lighting

A single lighting fixture that consists of two, 20-watt "cool-white" fluorescent tubes is suspended at approximately 15 cm above the tank surface. In addition, lighting fixtures over adjacent tanks (approximately 40 cm to either side) provide light. Light levels range from 2500-2800 lux. Lights are controlled by time switches and we often use a 13-hr light and 11 hr dark schedule for larval culture. During the dark phase of the schedule a low background light level of 40-90 lux is present, but it also is extinguished if experimental conditions warrant it.

Aeration

Air is provided by means of air pumps and airstones connected by plastic tubing to adjustable gang valves. Light aeration, with fine bubbles, is best during the egg incubation phase and during the first 2-3 days of larval culture, but the rate of bubbling is increased later to help to insure that food organisms remain well distributed throughout the rearing tank.

Maintaining the Tank

Water for rearing is drawn through our laboratory seawater system from Biscayne Bay. It is filtered through dacron filter wool and a 35 µm screen before being added to the tanks. At the outset of an experiment dense blooms of a seawater-adapted Chlorella sp. and Anacystis sp. that were isolated from Biscayne Bay are each added at a rate of 3.3 ml per 1000 ml of culture water. Phytoplankton cell densities in the concentrates that are added range from 9×10^6 to 3×10^7 per ml. The methods used to culture phytoplankton are given below under Phytoplankton Culture.

The role of the phytoplankton is still not completely understood but presumably metabolites from the fish larvae and their food organisms are utilized as a nutrient source by the algae, thus maintaining metabolite levels within acceptable limits (Alderson and Howell, 1973; Brick, 1974; Siddall, 1974; Cohen, Finkel and Sussman, 1976). The small Chlorella and Anacystis cells (1-5 µm diameters) are not purposely consumed by fish larvae and almost certainly are of little value as a direct source of nutrition. Nevertheless, the presence of the phytoplankton helps insure successful culture of larvae. Without it, survival is unpredictable and complete mortalities are frequent even when all other requirements of our technique have been met.

Phytoplankton blooms usually increase from 9×10^4 to 9×10^5 algal cells per ml in the rearing tanks during the first 4 days. Thereafter, blooms are maintained at high levels (1×10^5 to 3×10^5 cells per ml) by daily additions of the culture phytoplankton (as much as 2.5 ml per 1000 ml of larval rearing tank water) if the bloom begins to subside. This practice is followed until larvae begin to metamorphose, at which time the phytoplankton no longer seems critical. Actually, either the Chlorella or Anacystis is sufficient by itself, but we add both to our culture systems as standard practice. The Chlorella grows best at 22-26°C and the Anacystis grows well over the range 22-34°C.

Partial water exchanges are carried out beginning on the third day after hatching and on subsequent alternate days. These consist of a 10% renewal on day 3, a 15% renewal on day 5 and 20% renewals on days 7, 9, etc. Water is removed from the tank by a screened siphon to prevent loss of larvae and is replaced by adding 35 µm filtered seawater from the seawater system. We have not determined if this water exchange and replacement schedule is optimum. However, we have adopted it as standard procedure in all of our experiments, so that it is not a variable in any of our work.

Remaining system maintenance consists of controlling nominal food concentrations throughout the experiment. This is a time consuming but necessary task for predictable culture results. Details of the procedure are given below under Foods and Feeding.

Larvae

Larvae of many marine species can be reared using methods described in this report. Most of our research presently is focused on four species: bay anchovy (Engraulidae), lined sole (Soleidae), spotted seatrout (Sciaenidae) and sea bream (Sparidae). Table 1 is a list of species that we have cultured in signficant numbers by these methods.

Stocking Densities

Survival and growth of larvae are density dependent. Number of stocked eggs has been demonstrated to affect eventual survival and growth of larvae. The effects are more pronounced for some species, like sea bream (Houde, 1975), than for others, such as lined soles or bay anchovies (Houde, in press). Both survival and growth tended to be reduced at stocking densities greater than 2.0 per liter.

Stocking rates can be adjusted depending upon the requirements of a particular experiment. For example, bay anchovies can be stocked at densities as high as 32.0 per liter and expected survival at transformation will be in excess of 40% if food organism concentration is maintained at 1000 per liter or higher. Growth also is relatively good. Neither growth nor survival of bay anchovy is much reduced at 32.0 per liter stocking level compared to levels less than 2.0 per liter. On the other hand, sea bream stocked at 32.0 per liter and fed at 1000 per liter or higher food levels have survival rates of less than 10% at transformation and poor growth. This contrasts with survival rates of more than 70% and good growth when stock density is 2.0 per liter or less (Houde, manuscript).

For species that we have tested, consistently high survival rates and good growth rates can be achieved by our method if stocking rate does not exceed 2.0 per liter if food (consisting of copepod nauplii) levels are maintained at 1000 per liter or higher.

Culture Period

The time that larvae are cultured varies depending upon experimental objectives, but many of our experiments range from 12 to 25 days duration.

At 24°C and higher most species with which we work are metamorphosed or are transforming in that time period. Actually, most of the critical events in the culture period occur within the first 5-7 days after hatching and relatively little mortality occurs subsequently when culture conditions are adequate.

Sampling Larvae

Larvae are routinely sampled from the rearing tanks to obtain growth data and data on feeding. They are pipetted from the rearing tank and preserved in 5% formalin. To obtain food habit and selection data, larvae are quickly pipetted into ice-cold 20% formalin to kill them quickly before they can defecate or regurgitate food items. This technique is only partially successful for clupeoid larvae, but works well for other taxa. Larvae are preserved in 5% formalin after they have died.

For some experiments, such as feeding rate determinations, larvae must be transferred alive from the rearing tank to an experimental unit. This can be done successfully, even with delicate larvae like those of bay anchovy, by dipping larvae in small beakers from the rearing tnak and transferring them with small amounts (< 50 ml) of water to the experimental tank. Larvae handled in this manner usually begin normal feeding behavior within 5 min after transferral.

Foods and Feeding

Type of Food

Three foods are used in our culture experiments. Wild plankton, consisting mostly of copepod nauplii and copepodites, is the major food. The rotifer Brachionus plicatilis and nauplii of the brine shrimp Artemia salina also are used.

Wild plankton is collected in either 35 or 53 µm mesh plankton nets that we suspend in the tidal flow from a dock at the laboratory. Catches are graded into three size fractions before being used. The largest fraction is that retained on a 280 µm mesh. It consists mostly of adult copepods, barnacle nauplii, crab larvae and chaetognaths. We seldom use this size fraction as food, except for some experiments where larvae are grown past the transformation stage. The intermediate size fraction passes the 280 µm mesh, but is retained on a 110 µm mesh. Its major components are copepodites, large copepod nauplii and occasionally barnacle nauplii. Mean dry weight of organisms in this fraction is 0.51 µg (approximately equal 0.00286 cal.). We begin to feed this size food to larvae when they are large enough to consume it; for most species this occurs at 7-10 days after hatching. The smallest size fraction used as food passes the 110 µm mesh and is retained on either a 35 or 53 µm mesh. More than 95% of this fraction is either copepod nauplii or copepodites of 35-110 µm in breadth, except on uncommon occasions when tintinnids are abundant. Mean dry weight of these organisms is 0.12-0.15 µg (approximately 0.00067 to 0.00084 cal.). This fraction is the first food provided to larvae, usually on the second or third day after hatching when they can first feed.

Our rotifers are the California strain whose characteristics have been described by Theilacker and McMaster (1971). We culture the rotifers in our laboratory (see section below on Rotifer Culture). For the first feeding larval fishes, only rotifers passing a 110 µm mesh and retained on a 53 µm mesh are offered as food. Theilacker and McMaster (1971) gave the mean dry weight of a Brachionus plicatilis as 0.16 µg (equal 0.0008 cal.).

Brine shrimp nauplii that we hatch in aerated seawater originate from eggs collected in San Francisco Bay. Newly hatched nauplii are more than 350 µm in breadth and thus too large to be eaten by fish larvae of species that we rear when they begin to feed. Brine shrimp nauplii can be consumed when larvae have grown and we use this food in some experiments. Paffenhofer (1967) gave the dry weight of a newly-hatched brine shrimp nauplius as 1.65 µg (equal 0.00924 cal.).

Adequacy of the Foods

The wild plankton (copepods) is a good diet for larval fishes during the entire larval period. At suitable concentrations of wild plankton larval survival and growth are predictably good. Rotifers are an adequate diet and all marine fish larvae that we have tested (7 species) have survived to the transformation stage about as well as when wild plankton was fed. But, growth of larvae is relatively poor when rotifers are fed even when concenrations of rotifers are made available in quantities equivalent to wild plankton levels that provide good growth. Brine shrimp nauplii when fed to advanced larvae, produce variable results. Some larvae, such as lined soles, grow better on this food than on wild plankton. But, spotted seatrout larvae do not obtain good growth on brine shrimp nauplii and clupeoid larvae grow poorly when brine shrimp nauplii are the only food available.

Determining Food Concentrations

Most typical marine fish larvae are capable of searching a limited volume of water in a unit time. The number of contacts with food depends upon the concentration of food items in the rearing tank. Maintaining food concentrations at the nominal experimental concentration is an important part of the culture procedure.

Aliquots from a concentrated food organism suspension can be used to estimate the abundance of food in the concentrate and the volume of food suspension needed to adjust the food level of a rearing tank to a nominal concentration. Wild plankton and brine shrimp are concentrated near a light before being pipetted into the rearing tank. Rotifers are concentrated into a small volume of seawater by gently filtering them from their culture medium before they are added to the rearing tank. Using these methods only small quantities of media in which food organisms have been held are transferred to tanks with fish larvae.

Aliquots of 50 to 200 ml are pipetted from rearing tanks to determine their food concentrations. At nominal food concentrations of 1000 per liter or higher 50 ml aliquots are drawn, at 100-1000 per liter food levels 100 ml aliquots are used and at food levels less than 100 per liter 200 ml aliquots

are used and at food levels less than 100 per liter 200 ml aliquots are used. The mean count of two such aliquots usually is sufficient to estimate the mean food concentration within ± 20%. Food concentrations are estimated in the rearing tanks from 3-6 times daily. When necessary, food is added to tanks to adjust concentrations to nominal levels.

Food Concentrations

Although requirements vary among species, a food concentration of 1000 per liter copepod nauplii will give high percentage survival (> 40%) and good growth for all species that we have tested. Slightly better survival and slight improvements in growth sometimes occur at food levels above 1000 per liter, but in our experience no species require food organisms of the kinds that we used at 10,000 per liter levels. Some larvae of most species that we have tested can survive to metamorphosis when copepod nauplii concentrations are only 10-50 per liter. For experiments where survival and growth rates must be predictable, a food concentration on the order of 1000 per liter is appropriate.

Higher concentrations of rotifers are required to give growth that is almost as good as growth on copepod nauplii, but survival rates are nearly the same for larvae fed equivalent concentrations of either rotifers or copepod nauplii. Brine shrimp nauplii are only fed to advanced larvae with well-developed swimming ability. We have no estimate of required brine shrimp concentrations for growth and survival but fish larvae at this stage do not require high concentrations (e.g., 1000 per liter) for good growth.

Phytoplankton Culture

We routinely culture the planktonic algae *Chlorella* sp. (Chlorophyceae) and *Anacystis* sp. (Cyanophyceae) at room temperatures of 20-26°C in racks illuminated with vertical light banks of 40-watt "cool-white" fluorescent lamps. Both species are cultured in liquid bleach-disinfected 19-liter glass carboys. After disinfecting, carboys are emptied, filled with tap water and set aside for about 12 hrs to remove traces of chlorine. Seawater filtered through a 35 µm mesh screen is adjusted to about 30°/oo salinity, vigorously aerated and pasteurized at 70-80°C for about 20 min using a 500-watt immersion heater. We use a timer to control the immersion heater which simplifies the pasteurization process. Pasteurized seawater may be kept in capped carboys up to 3 days at temperatures below 26°C without noticeable effect upon new phytoplankton cultures.

Medium "f/2" (McLachlan, 1973) is used for both *Chlorella* and *Anacystis*. Two grams urea and 0.3 g sodium phosphate monobasic per 19-liter carboy are added to the "f/2" medium for *Anacystis* cultures. *Anacystis* maintains its exponential growth phase for a longer period when urea and sodium monophosphate are added to the "f/2" medium.

Five hundred milliliter stock cultures of *Chlorella* are allowed to attain high densities in one liter Erlenmeyer flasks. These stock cultures are usually ready for use in 10-15 days, but may be incubated as long as two months when kept at temperatures below 26°C. We have not successfully

maintained Anacystis in small volume stock cultures. We inoculate with one liter of 35 µm filtered Anacystis from previous 19-liter cultures. For our xenic planktonic algae cultures rapid growth is important. Vigorous aeration prevents the algal cells from clumping and promotes rapid growth. Carbon dioxide is not metered into the air supply.

Ukeles (1973) reported that exponential growth begins about 3-5 days after inoculation. We usually use our planktonic algae between 4-21 days after inoculation.

Rotifer Culture

The rotifer Brachionus plicatilis is regularly maintained by inoculating 10-14 day old Chlorella cultures with 500-1000 ml of Brachionus stock cultures. Cultures are kept illuminated by banks of 40-watt "cool-white" fluorescent lights and maintained at 23-25°C. About 7-10 days after inoculation the density of Brachionus is about 200-300 per ml and such cultures are used to feed larval fish. Chlorella is added ad libidum to keep the rotifers well-fed. Rotifer stock cultures are maintained in 2800 ml Fernbach culture flasks. About 500 ml of an exponentially growing Chlorella culture is added to a Fernbach flask and a 20 ml inoculum from another dense rotifer stock culture added. Ciliates may periodically contaminate stock and carboy cultures. Rotifers from ciliate-contaminated cultures appear healthy but the rotifers' exponential growth phase may be suppressed due to the contaminants.

TABLE 1. LIST OF MARINE FISHES THAT HAVE BEEN CULTURED FROM EMBRYO THROUGH LARVAL STAGES USING METHODS DESCRIBED IN THIS REPORT. SPECIES NAMES FOLLOWED BY AN ASTERISK HAVE READILY AVAILABLE EGGS AND HAVE BEEN CULTURED ON MANY OCCASIONS AT RSMAS.

Clupeidae	
Brevoortia smithi	yellowfin menhaden*
Harengula jaguana	scaled sardine*
Opisthonema oglinum	Atlantic thread herring
Sardinella anchovia	Spanish sardine
Engraulidae	
Anchoa hepsetus	striped anchovy*
Anchoa lamprotaenia	bigeye anchovy*
Anchoa mitchilli	bay anchovy*
Exocoetidae	
Hemiramphus brasiliensis	ballyhoo
Carangidae	
Oligoplites saurus	leatherjacket
Trachinotus carolinus	Florida pompano
Trachionotus falcatus	permit
Pomadasyidae	
Haemulon plumieri	white grunt*
Orthopristis chrysoptera	pigfish*
Sparidae	
Archosargus rhomboidalis	sea bream*
Diplodus holbrooki	spottail pinfish
Sciaenidae	
Bairdiella chrysura	silver perch
Cynoscion nebulosus	spotted seatrout*
Equetus acuminatus	high-hat
Leiostomus xanthurus	spot
Micropogon undulatus	Atlantic croaker
Ephippidae	
Chaetodipterus faber	Atlantic spadefish*
Labridae	
Lachnolaimus maximus	hogfish
Mugilidae	
Mugil curema	white mullet*
Sphyraenidae	
Sphyraena borealis	northern sennet

Scombridae	
Euthynnus alletteratus	little tunny
Scomber japonicus	chub mackerel
Triglidae	
Prionotus sp.	searobin
Bothidae	
Paralichthys albigutta	Gulf flounder
Soleidae	
Achirus lineatus	lined sole*
Gymnachirus melas	naked sole
Ostraciidae	
Lactophrys quadricornis	scrawled cowfish

APPENDIX A

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APPENDIX B

OBTAINING EMBRYOS FROM ADULT SPOTTED SEATROUT

Obtaining Adults

Adults were caught on hook and line, brought to the laboratory and held outdoors in 2 m^3 tanks which held 1 m^3 of seawater that was replaced at 30 liters per minute. Individuals were segregated by sex. They were fed *ad libitum* on live pink shrimp (*Penaeus duorarum*) and mullet (*Mugil* sp.) flesh. Females that were to be spawned were held in the tanks for periods no longer than two weeks after capture.

Determining Sex

Disposable Pasteur pipettes, 15.25 cm (6 in) long, with 5.08 cm (2 in) flame-rounded tips, were used as glass catheters. The catheters were attached to flexible tubing through which oral suction was applied to draw a biopsy plug of ovarian tissue or hydrated milt into the tip of the pipette from MS-222 anesthetized fish. The tip of the catheter was inserted 10 mm into the sperm duct to check males. If no milt was drawn, the catheter was fully inserted and a biopsy plug was withdrawn to determine sex of the fish. A 132 mg per liter solution of tricaine methane sulfonate (MS-222) in seawater was judged to be a satisfactory anesthetic. Rapid induction of anesthesia reduced expulsion of ovulated eggs by females that were ready to spawn.

Hormone-Induced Ovulation

Well known techniques using human chorionic gonadotropin (HCG) injections (Stevens 1966, 1970; Shehadeh and Ellis 1970; Haydock 1971; Kuo *et al*. 1974; Hirose *et al*. 1979) were used to induce oocyte maturation and hydration in spotted seatrout. Spotted seatrout can spawn year round in south Florida (Stewart 1961; Jannke 1971). They are certainly able to spawn for 8 months of the year in Biscayne Bay, usually from late March through October. Pelagic eggs were common in Biscayne Bay, but naturally spawned eggs were not always easily separated from eggs of other species with similar eggs. Artificial spawning of adult seatrout eliminated the identification problem and provided large quantities of nearly synchronously developing embryos.

A 1 cc disposable tuberculin syringe with a 25-guage 15.9 mm (5/8-inch) long hypodermic needle was used to adminster lypophilized HCG dissolved in Holtfreter's saline. To successfully induce oocyte maturation and

ovulation, females were injected when biopsied ovarian fragments revealed oocytes within the follicles $\geq$ 0.40 mm diameter. But, the technique was most successful when oocytes were greater than 0.45 mm diameter. A single injection of 1 to 1.5 IU HCG per gram body weight was injected intramuscularly into the hypaxial musculature beside the first dorsal fin. Injected females were kept in a water table until they ovulated. An anesthetic, 132 mg of MS-222 per liter of seawater, was used before the HCG injection and before every gonad biopsy. Most females ovulated within 28 to 34 hours after the injection. A biopsy at 24 hr after injection indicated if the oocytes were maturing and enabled estimation of subsequent biopsy times. Ovulated oocytes in the lumen of the ovary remained viable for about 60 minutes. Ovulated eggs usually were removed from the female within 30 minutes after they were detected in a biopsy. Eggs were extruded from the ovary by applying pressure to the abdomen or they were aspirated into a catheter. A catheter was preferred when batches of eggs free from faecal, mucous, and blood clot contamination were required. Females survived hormone-induced spawning with no apparent ill effects.

Males were mature throughout the long spawning season but they were injected with the same HCG dose as given to the females. The injection appeared to help hydrate the milt in the testes and facilitated the aspiration of milt into the catheter.

Fertilization and Cleaning of Eggs

Eggs were fertilized by the dry method in a clean dry dish. Several drops of milt were placed in the dish, then the eggs were added and finally seawater to activate the sperm. This technique was selected because large amounts of milt could not be extracted from each male, and because fish sperm generally remain viable as long as they are not activated by water.

Eggs were rejected if it appeared that less than 50% were fertilized. This decision was easily made because HCG-induced spawnings produced egg batches which were visibly good or bad. Zygotes were kept in the fertilizing dishes and placed in constant temperature water baths. They could be transferred to other containers or tanks without inducing mortality or deformities if the transfer was accomplished before germinal ring development or after gastrulation. Zygotes surviving gastrulation and showing normal development were selected for experiments.

APPENDIX B

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